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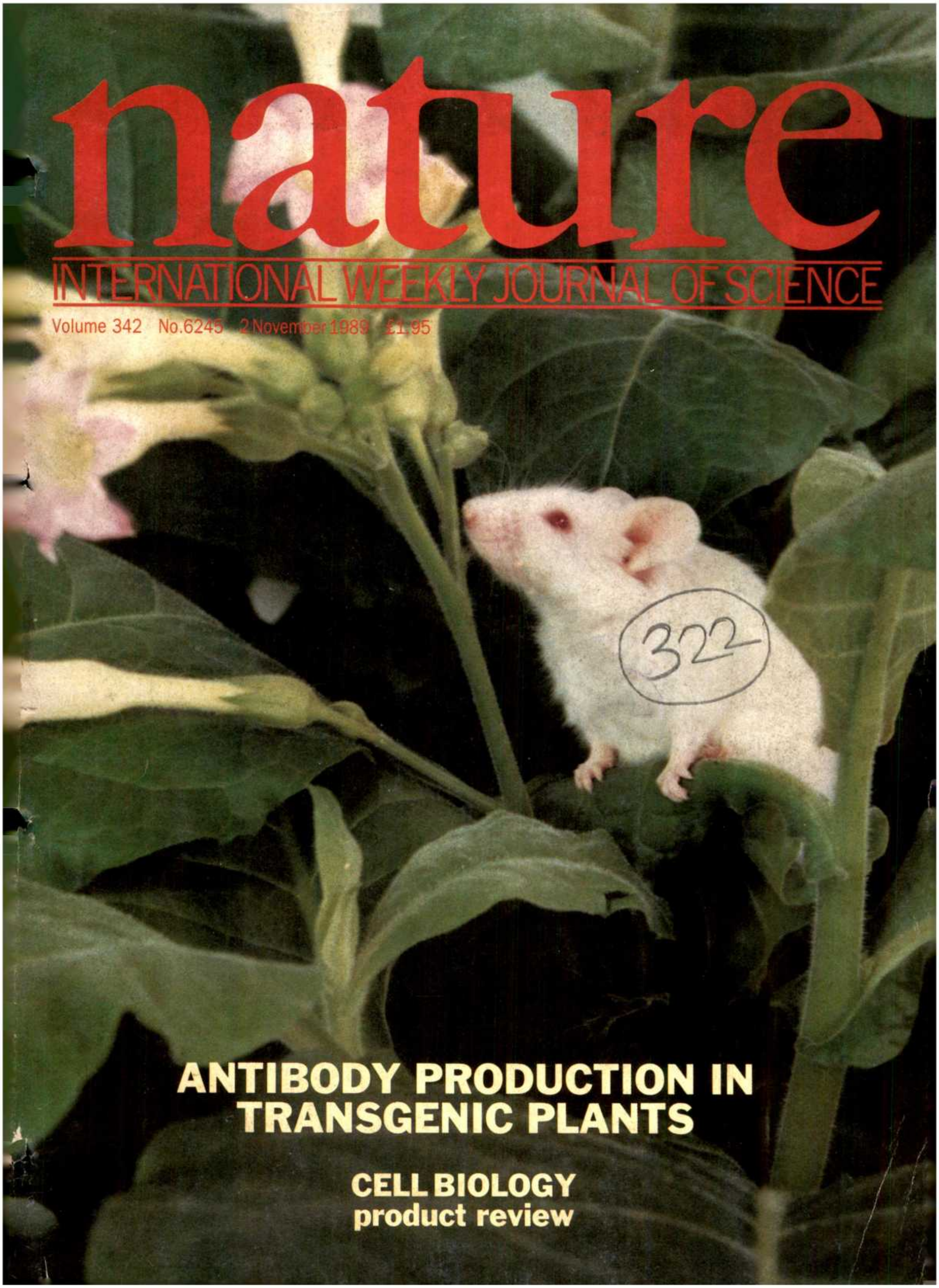

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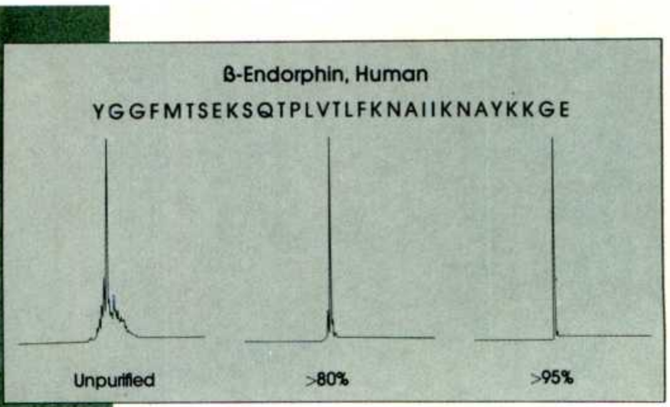

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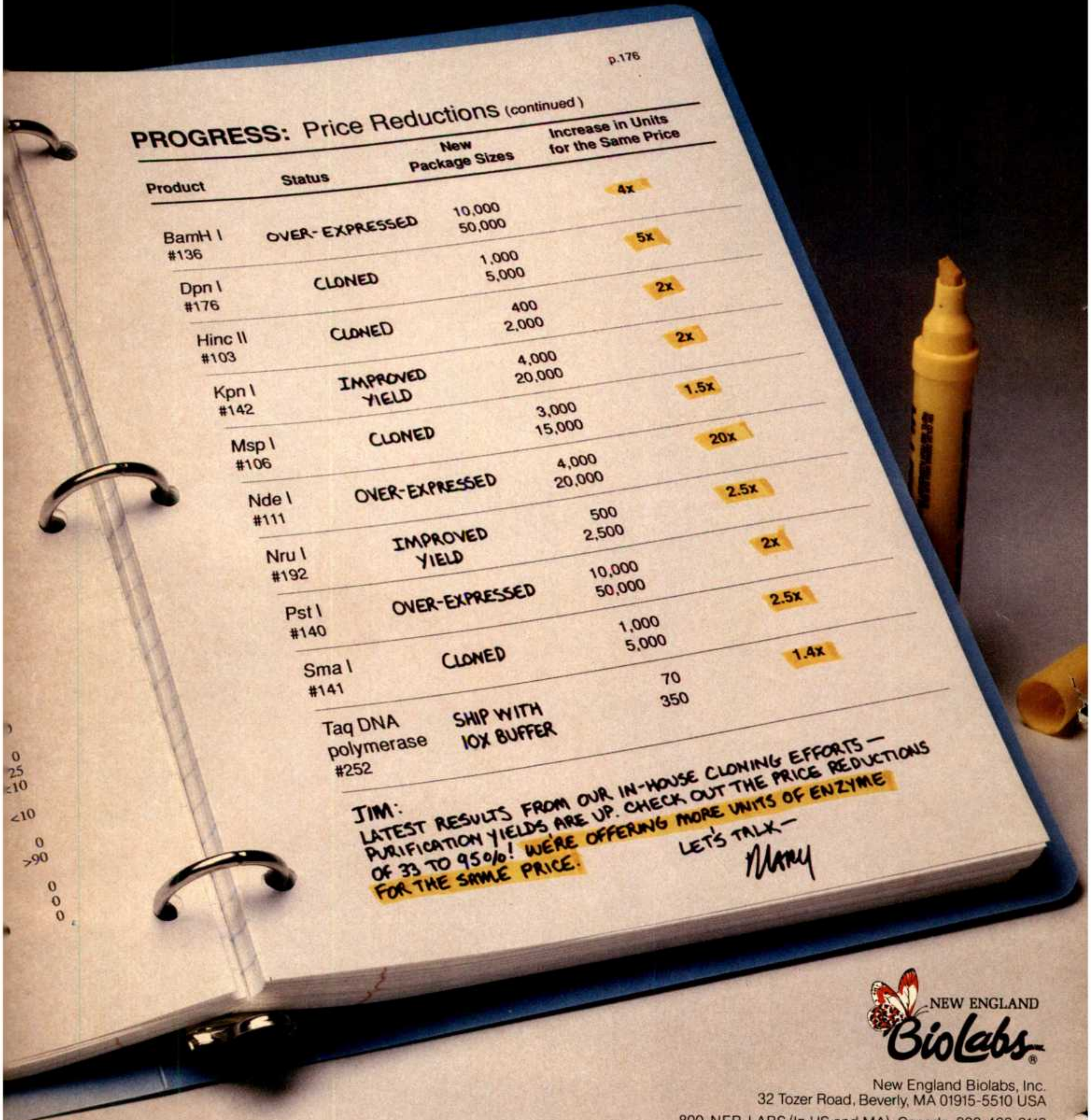

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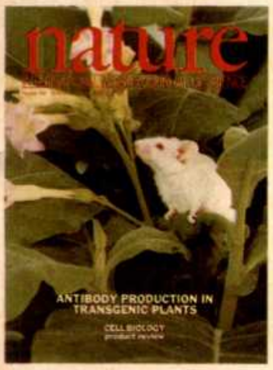
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ANTIBODY PRODUCTION IN TRANSGENIC PLANTS
CELL BIOLOGY product review

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2 November 1989

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◀ Transgenic plants bearing genes for immunoglobin light and heavy chains are able to assemble functional antibodies. The expression of antibodies in plants could be of both scientific and practical value. The cover shows a BALB/c mouse and transgenic tobacco plant. See page 76.

THIS WEEK

Oil shock

Petroleum-like deposits associated with hydrothermal vents beneath the Gulf of California are shown by radiocarbon dating to be geologically 'young', less than 5,000 years old, but to have a structure similar to conventionally exploited crude oils which are formed over much longer timescales. Page 65.

Sunspot data eclipsed?

A new computer model of the global troposphere suggests that the internal dynamics of the atmosphere can produce variability in the period range often attributed to solar influences. 'External' factors need not be invoked to explain variations, so 30 years of studies of sunspot–weather correlations may be due for reappraisal. Pages 53 and 15.

Muscle-bound

The nematode protein 'twitchin' is a member of a class of very large muscle proteins, and plays an important part in the control of muscular contractility. The sequence of this protein reveals a simple pattern of internal repeats based on sequence motifs with surprising similarities to immunoglobulins and cell adhesion molecules. Page 45.

The $InsP_3$ receptor

The cloning and sequencing of the cDNA of glycoprotein P_{400} from cerebellar Purkinje neurons provides insights into the structure of the receptor for inositol 1,4,5-trisphosphate ($InsP_3$), second messenger in many cells including neurons. Article, page 32. On page 87 it is shown that $InsP_3$ promotes calcium transport into vesicles reconstituted with its receptor protein, suggesting that $InsP_3$-stimulated release of intercellular calcium is mediated by ligand-gated calcium channels. See also page 16.

Level pegging

Lake Tanganyika, the second deepest lake in the world and probably one of the oldest, has been subject to marked lake-level fluctuations. New datings, derived from diatom analysis of cores extending back beyond the limits of the radiocarbon timescale, pin down the depth variations more accurately than previously possible, allowing comparisons with global sea level and climate trends. Page 57.

Decisive step

The phosphorylation state of the product of the cell-cycle gene *cdc2*, protein kinase pp34, directly determines entry into mitosis, a key step in regulating the eukaryotic cell cycle. Article, page 39, News and Views page 14.

Fifth force not needed

Anomalous gravity measurements, previously attributed to the existence of a mysterious 'fifth force', can be explained more simply by invoking geologically reasonable density variations underground. See page 29.

Strategy for repair

DNA repair in *E. coli* occurs preferentially on the transcribed strand during active transcription of the *lac* operon, suggesting that nucleotide excision and repair are coupled, page 95.

Black Sea bugs

The existence of high concentrations of bacteriochlorophylls in the chemocline of the Black Sea suggests that anaerobic photosynthesis could contribute to primary production in the Black Sea carbon cycle. Page 69.

Bang up to date

The notion that Big Bang cosmologies imply that time 'began' is challenged, page 23.

Guide to Authors

Facing page 766 in 26 Oct. issue.

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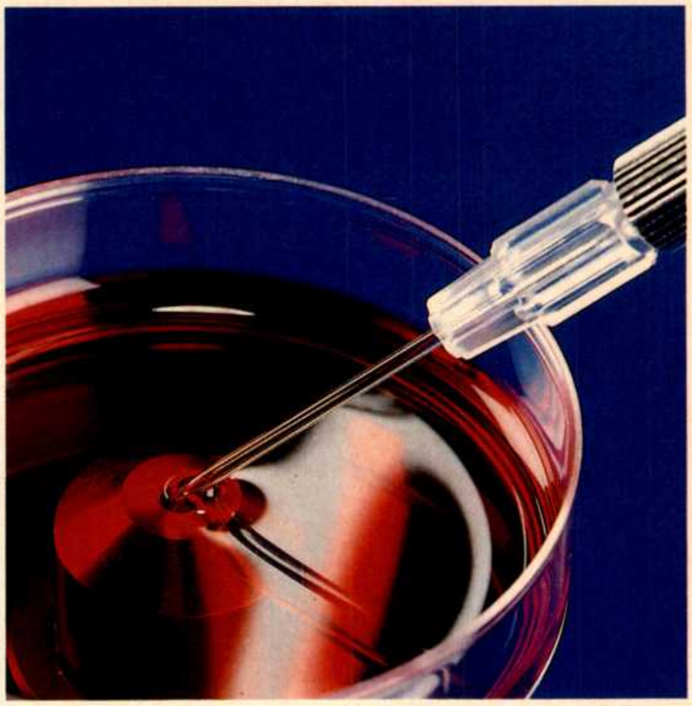

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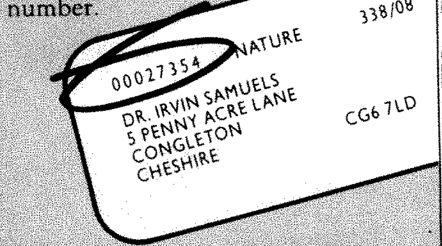

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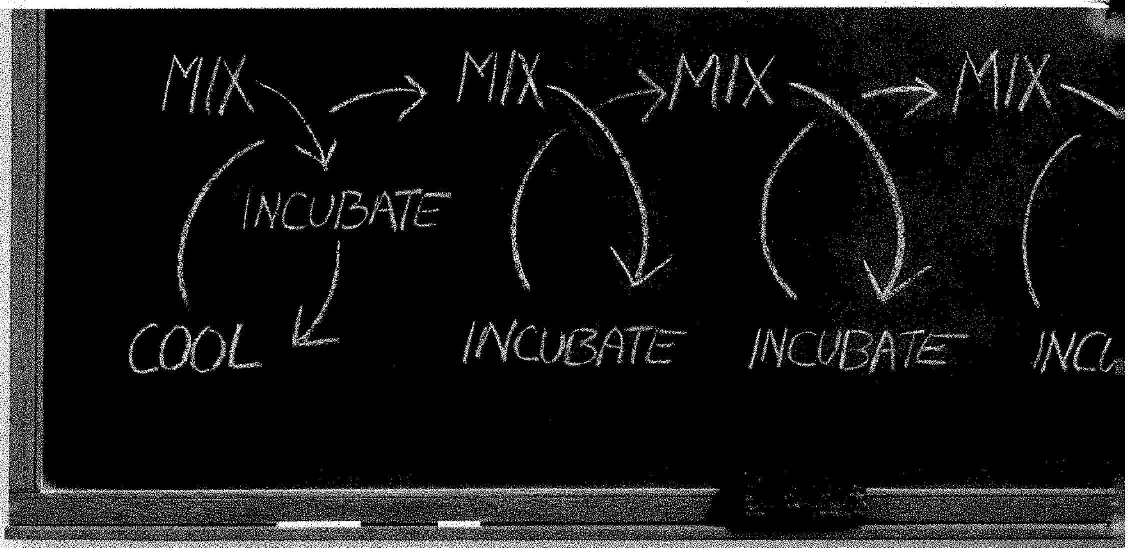

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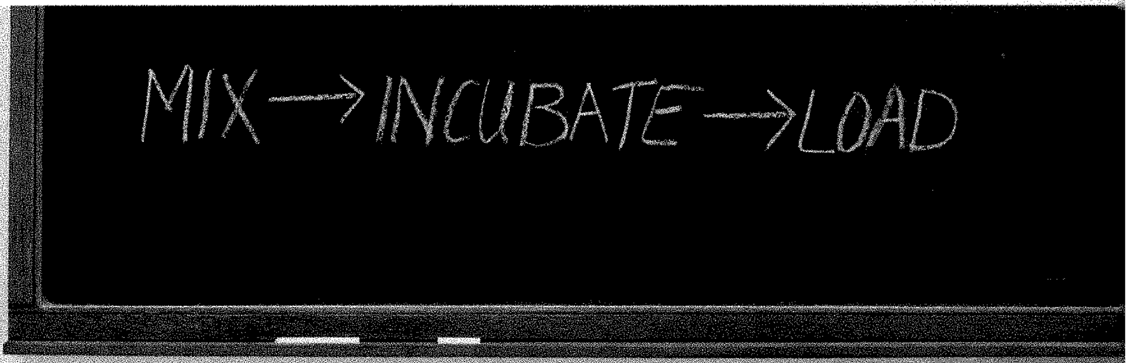

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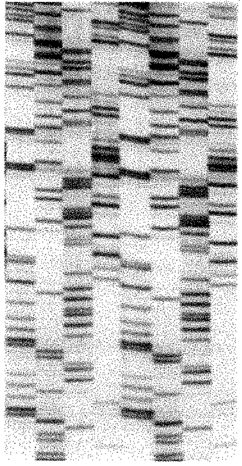

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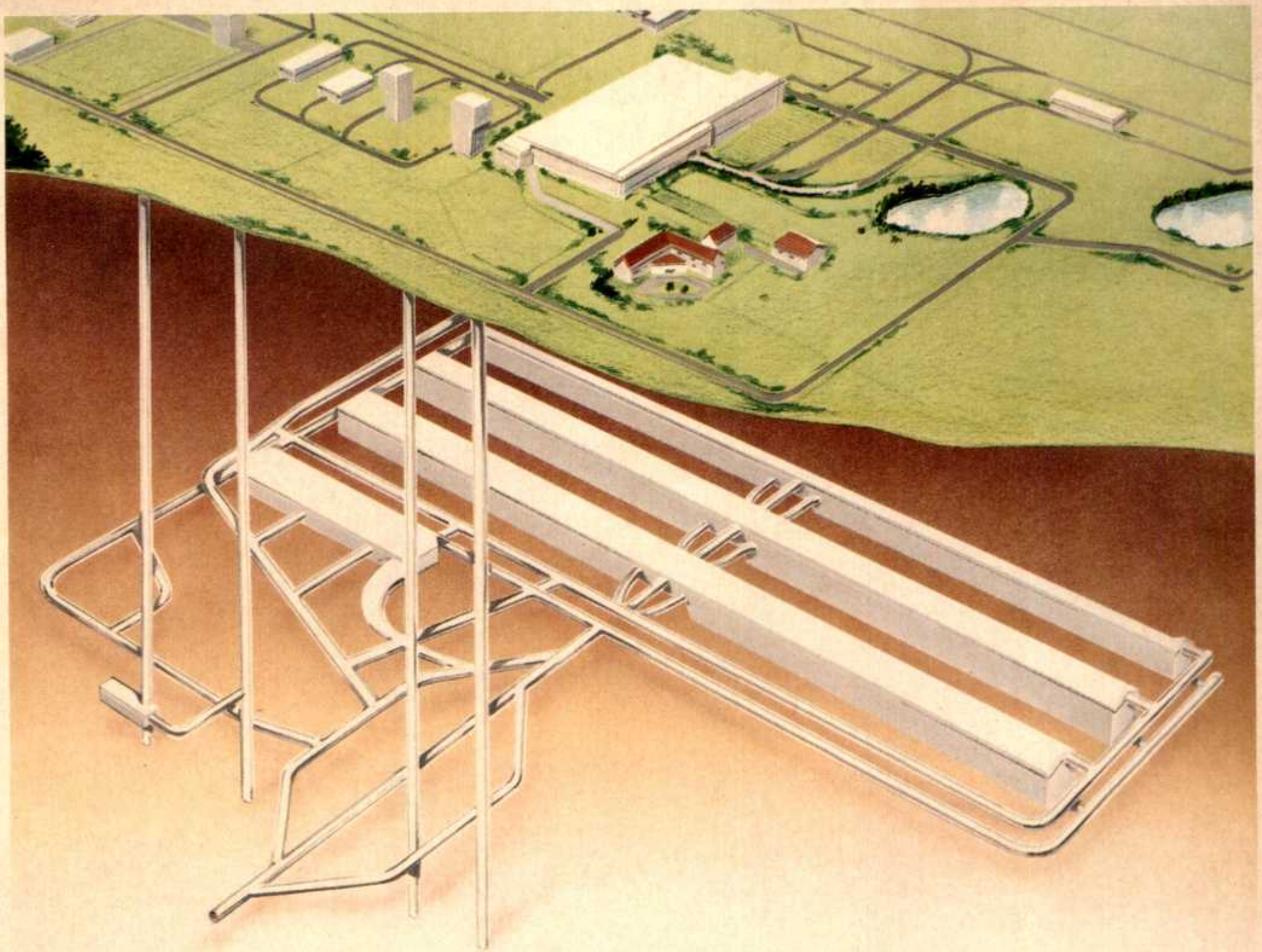

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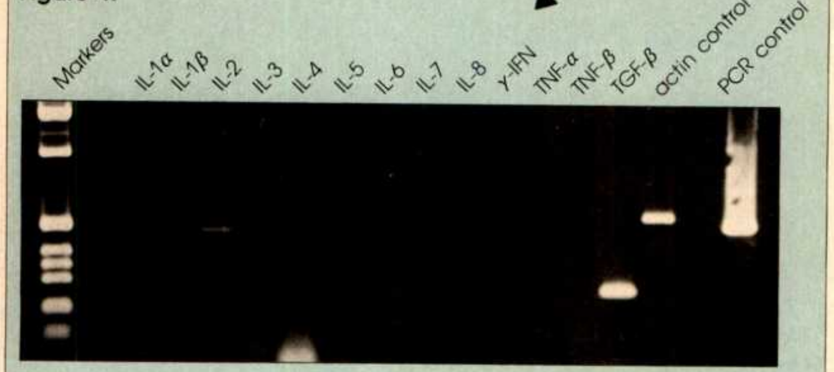

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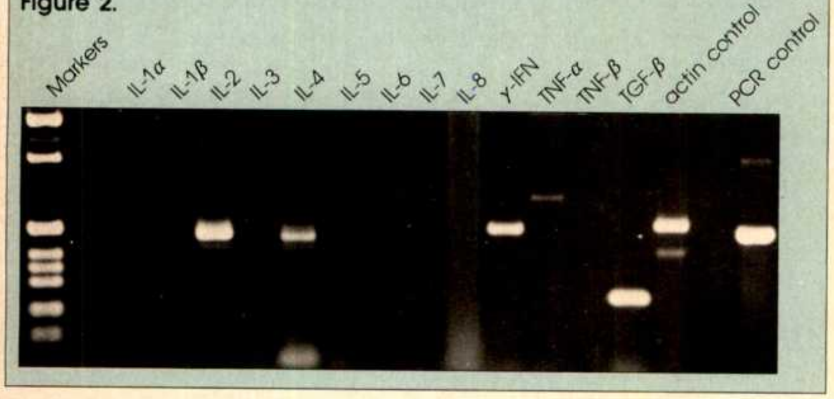

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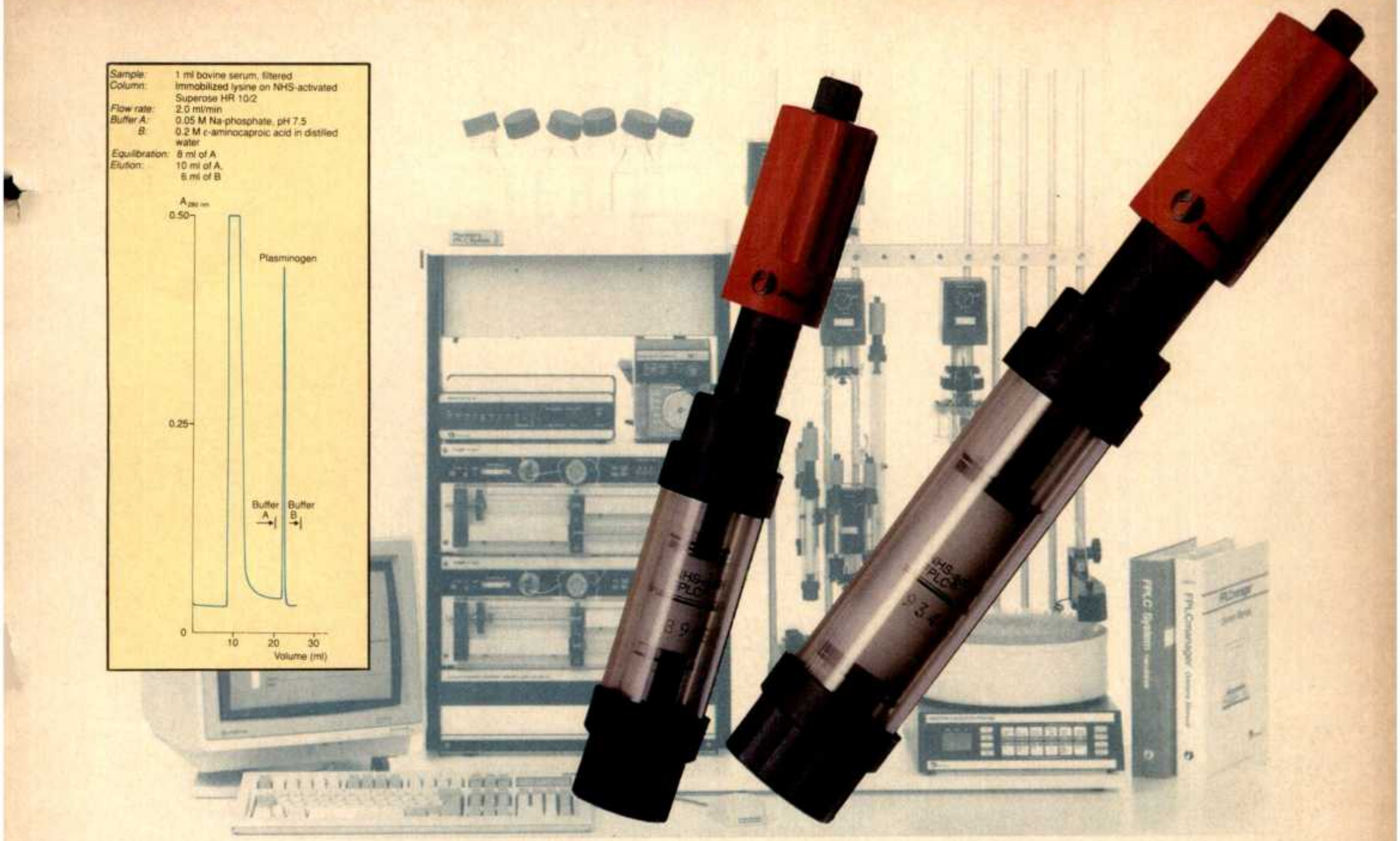
Sample: 1 ml bovine serum, filtered
Column: immobilized lysine on NHS-activated Superose HR 10/2
Flow rate: 2.0 ml/min
Buffer A: 0.05 M Na-phosphate, pH 7.5
B: 0.2 M ε-aminocaproic acid in distilled water
Equilibration: 8 ml of A
Elution: 10 ml of A, 6 ml of B
A 280 nm
0.50
0.25
0
Plasminogen
Buffer A
Buffer B
10
20
30
Volume (ml)

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Pharmacia LKB, established leader in the field of affinity gels, introduces yet another first: N-hydroxysuccinimide (NHS)-activated Superose®, for high performance affinity chromatography with FPLC.

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Our affinity gels show low non-specific interaction and negligible ligand leakage. They are based on Superose 12, the well-known high performance agarose hydrophilic matrix (13 μm), with high chemical stability over a broad pH range.

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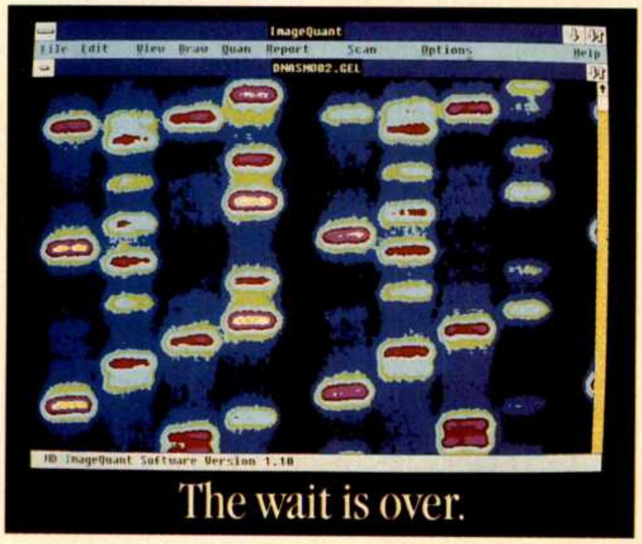
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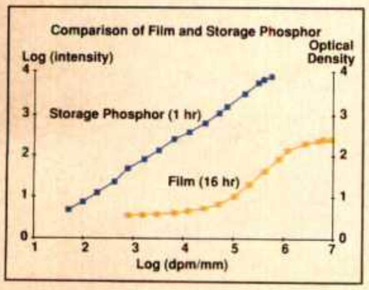

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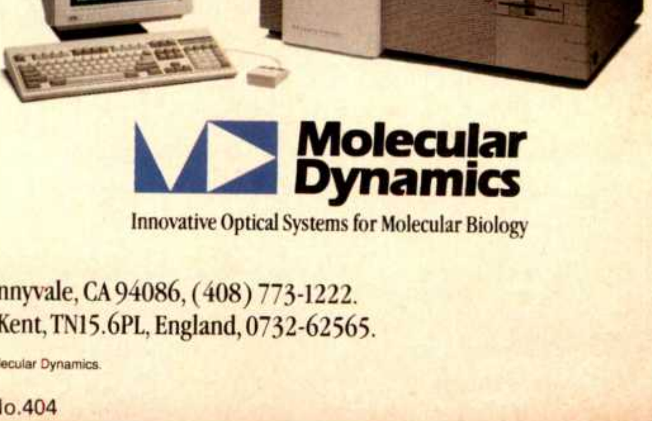

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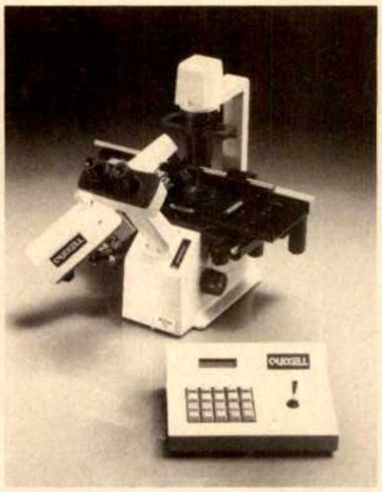

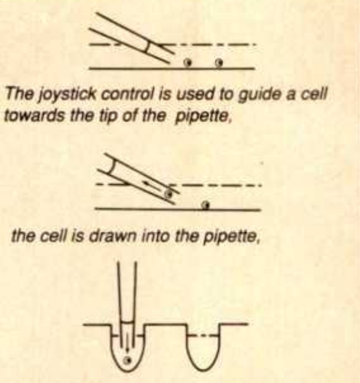

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- Sensitivity :
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  Background : 1.7 cpm per cm^2
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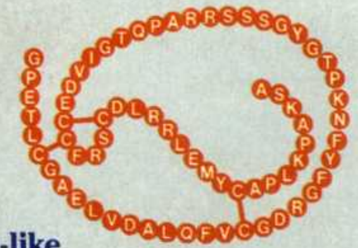

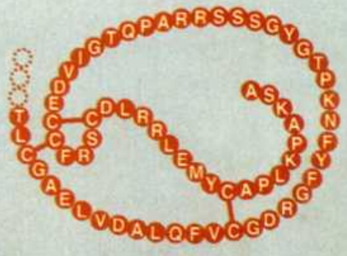

Insulin-like Growth Factor I (IGF-I)

IGF-I is a 70-amino acid long peptide hormone involved in growth and metabolism. It is one of the most important growth-regulating hormones active after birth; the secretion of IGF-I is influenced both by serum growth hormone levels and by nutritional status.

IGF-I has been shown to influence the growth and differentiation of many types of animal cells, including: ***human*** chondrocytes and adrenergic neuroblastoma cells; ***mouse*** late-stage erythroid precursor cells and mammary epithelial cells; and ***rat*** myoblasts, oligodendrocytes, calvaria cells and thyroid cells.

Specifications

Purity:	>95% by HPLC, gel electrophoresis and amino acid composition
Sp. activity:	>14,000 U/mg by radioreceptor assay using placenta membranes
Quantity:	10 μg, 50 μg, 100 μg, 1 mg, 10 mg and 50 mg.

Brain IGF

Brain IGF is a truncated form of IGF-I (lacking the N-terminal tripeptide) isolated from human foetal and adult brain.

Brain IGF may be important in the growth and metabolism of the central nervous system. It is four times more potent than IGF-I in stimulating DNA synthesis in rat foetal brain cells, and reacts more strongly than IGF-I in cross-reaction on IGF-I receptors in human foetal and adult brain membranes.

Specifications

Purity:	>90% by HPLC, gel electrophoresis and amino acid composition
Sp. activity:	>14,000 U/mg by radioreceptor assay using placenta membranes
Quantity:	10 μg, 25 μg, 50 μg, 100 μg, 1 mg, 10 mg and 50 mg.

Polyclonal antibodies (globulin fraction) to human IGF-I

Polyclonal antibodies to IGF-I have been prepared in New Zealand White rabbits using as immunogen a fusion protein comprising either IGF-I or the C-terminal residues 57–70 of IGF-I linked to a synthetic IgG-binding protein.

IGF-I and Brain IGF react equipotently to antibodies to the C-terminal peptide of IGF-I, whereas Brain IGF is less potent (77%) than IGF-I in cross-reacting to antibodies to whole IGF-I.

Specifications

Titre:	In an immunoradiometric assay, a titre of 1:4,000 for anti-IGF-I and 1:7,000 for anti-IGF-I C-terminal residue
Presentation:	1 ml, freeze-dried from phosphate-buffered saline

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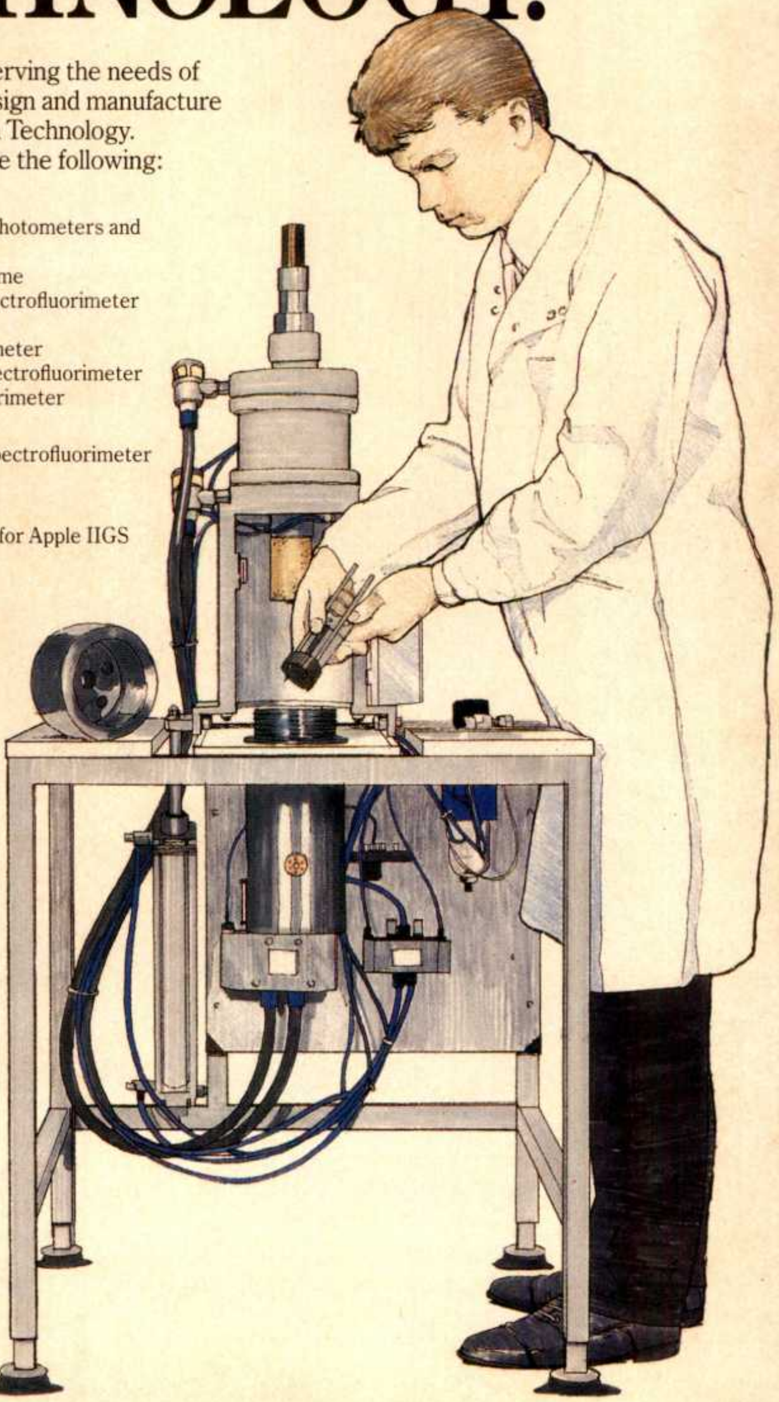

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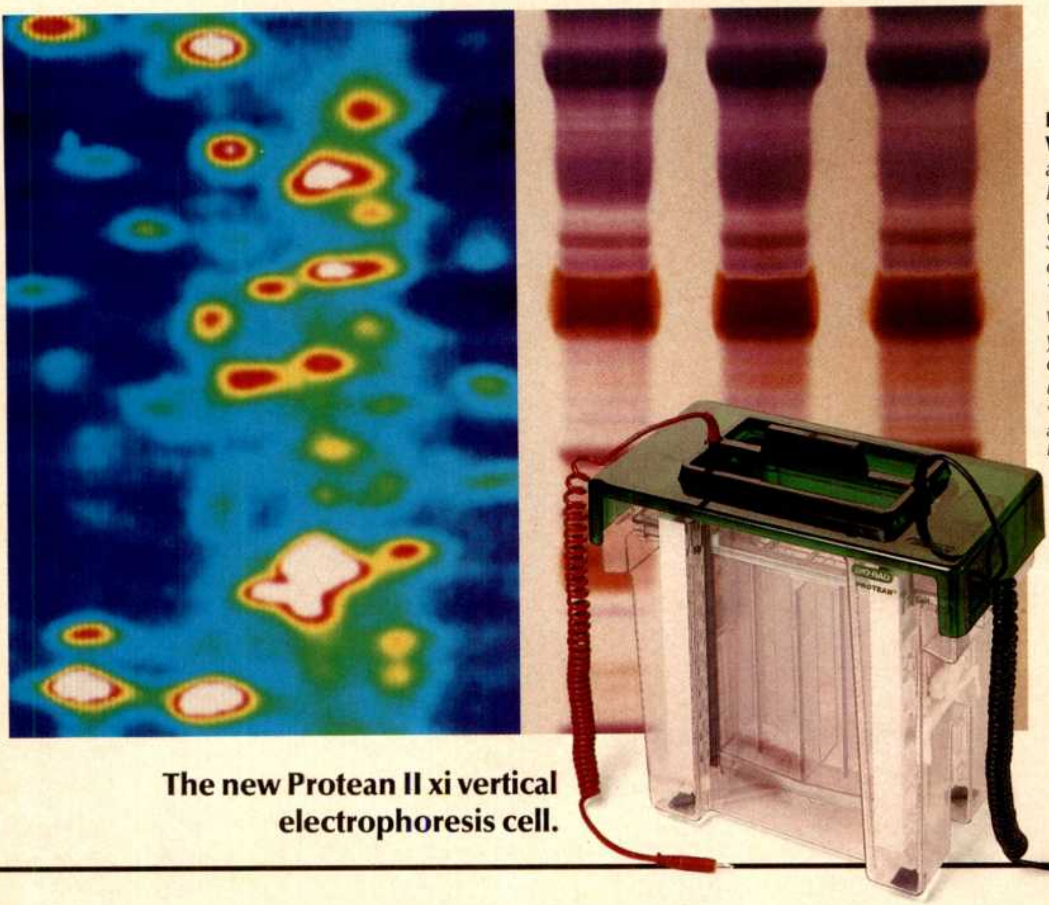

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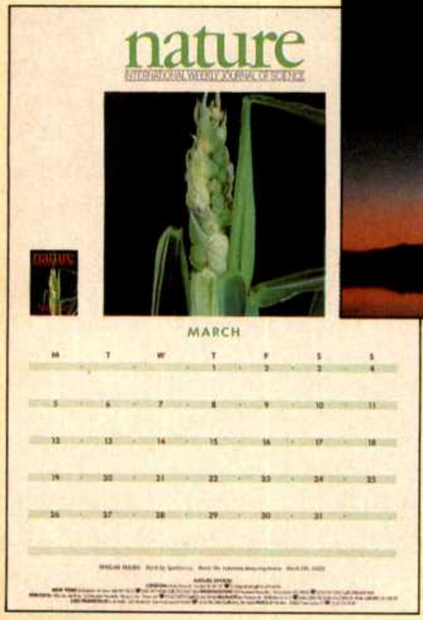

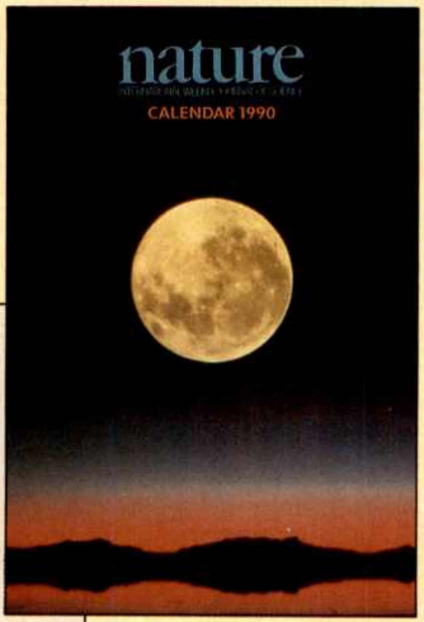

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By Michael B. Yarmolinsky.

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Multiple Determinants of Eukaryotic mRNA Stability. By Don W. Cleveland and Tim J. Yen.

HIV Trans-Activation and Transcription Control Mechanisms. By Katherine A. Jones.

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A review of the 5th Annual Meeting on Oncogenes; June 27-July 1, 1989; Frederick, MD, USA. By Sharon L. Cross.

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Review of A Forefront Symposium on the Organization of Membrane Polarity in Epithelial Cells; July 2-6, 1989; Arolla, Switzerland. By David G. Warnock, Bernard C. Rossier, and Jean-Pierre Kraehenbuhl.

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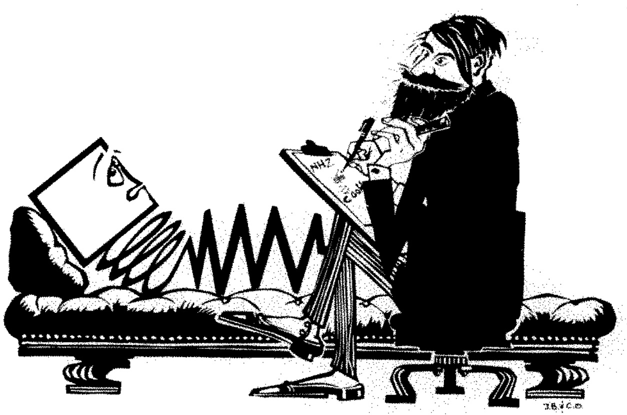

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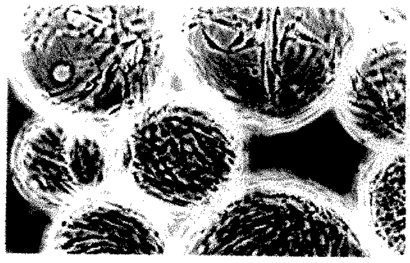

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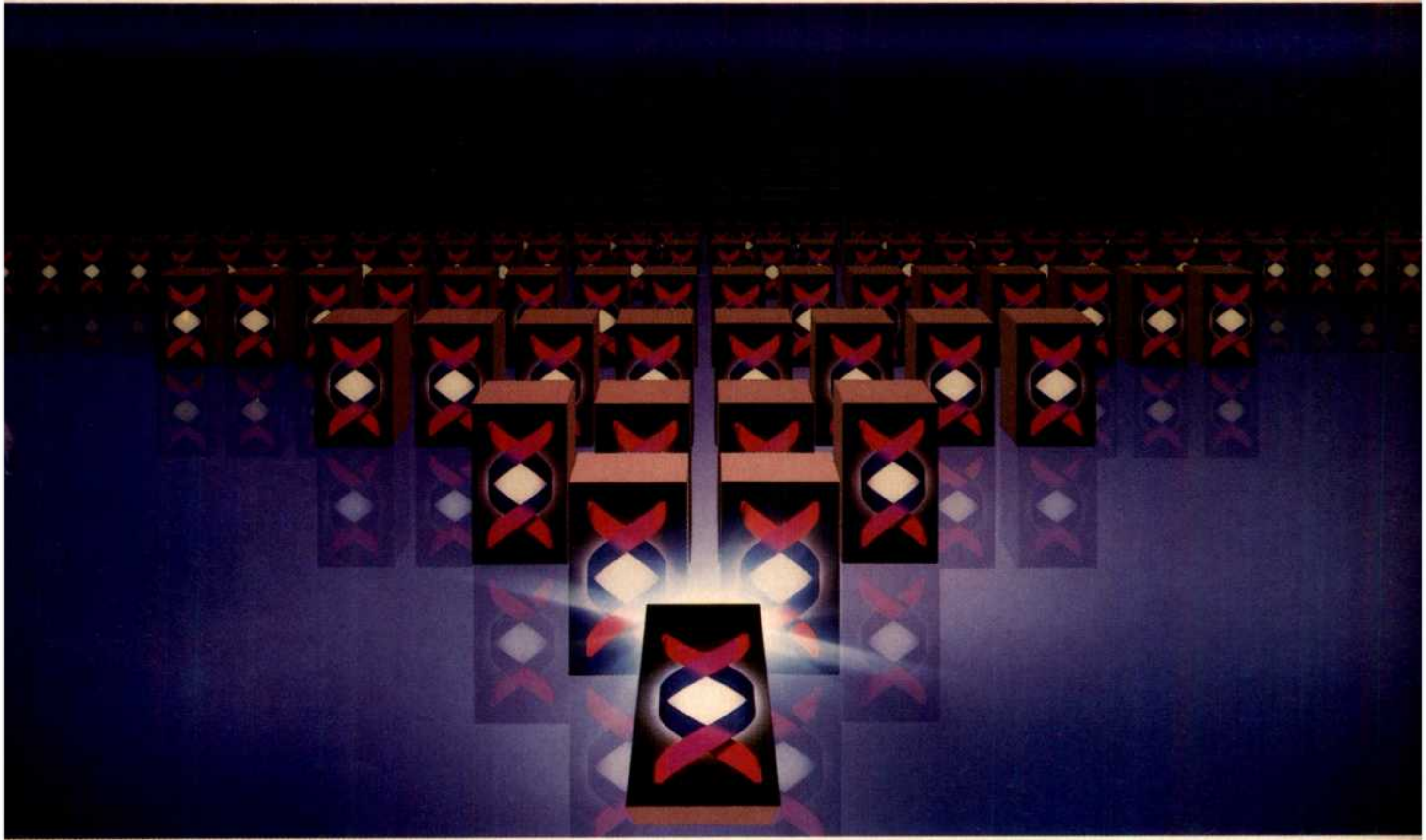

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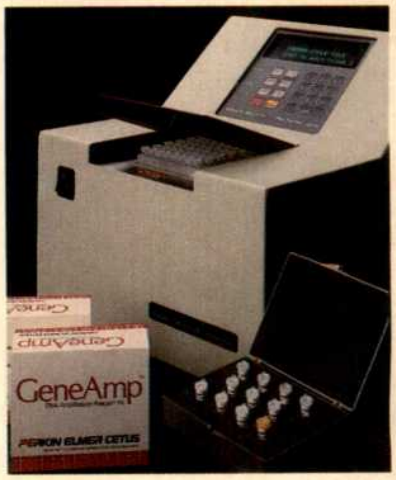

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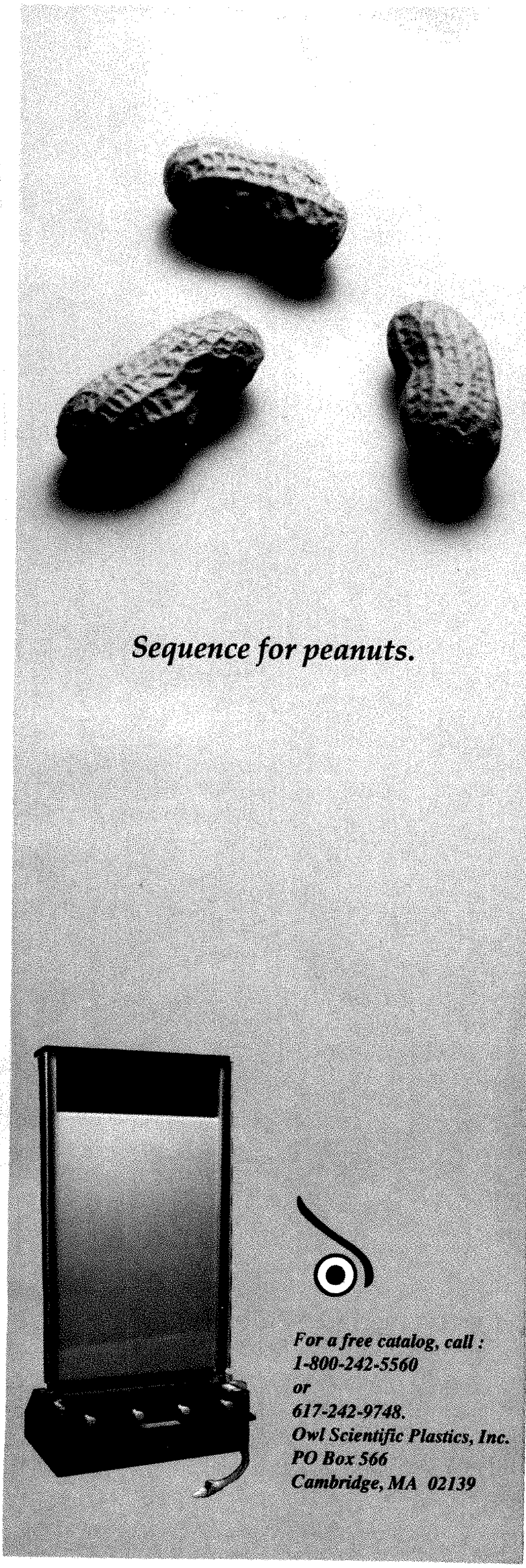

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James Baker's brave new world

The US Secretary of State is rightly cock-a-hoop about the prospects for arms control in the near future, but his vision of what follows is incomplete. But it is not too soon to be planning for the post-strategic world.

THE US Secretary of State, Mr James Baker, broke new ground on arms control in his speech in San Francisco last week. Telling how the prospects for far-reaching arms control agreements have been brightened in the past few years, he argued for paying attention now to the arrangements that will be necessary when the agreements now being negotiated have been signed and ratified. At this stage, Baker is doing little more than to think aloud, but it is radical thinking. Can he carry the rest of the US administration with him?

The list of agreements to which lawyers are putting the finishing touches is impressive. That on strategic arms has been made possible by the Soviet government's willingness to decouple the prospective agreement on strategic weapons from the Strategic Defense Initiative (SDI). That, Baker says, will be pursued within the confines of the Anti-Ballistic Missile Treaty. The objective, now, is to eliminate first-strike weapons. On conventional weapons, the superpowers seem to be moving towards an agreement based on the limitation of offensive weapons. A chemical weapons agreement is also almost within sight — the United States will destroy 80 per cent of its stocks on signature, and the Soviet Union will reduce its stocks to the same level, stocks will be reduced by a further factor of ten within eight years of a multilateral treaty and all chemical weapons will be destroyed when all states capable of making them have joined the treaty. It is a breathtaking prospect.

Is it likely to become a reality? Baker's new thinking, as *perestroika* would have it, takes the form of an open calculation of the chances. "We want *perestroika* to succeed", he says; "it would be folly indeed to miss this opportunity." But what if *perestroika* fails, and if the warp of history is rolled back? Then there will be arms control agreements in place, which the Soviet Union will be able to disown only with great difficulty, even risk. And, by implication, arms control agreements may even help *perestroika*. So far, so good.

What follows? One ingredient of the regime to come, in Baker's view, is the strategic *glasnost* provided by the arrangements for verification being written into the arms control treaties. Then, he says, there will be an opportunity also to deal with issues such as the proliferation of missile-making capacity, when the United States and the Soviet Union will be able to make common cause (as, fair play, they have done from the start on nuclear weapons). That, he says, will also be the time to embark on the "institutionalization of a safer world".

But need it wait that long? If *perestroika* has brightened the outlook, it has also deepened some uncertainties, not only in Central Europe, but in the Middle East, the Far East and in South-East Asia. What will happen if Hungary applies to join the European Communities, or seeks to renew its old alliance with Austria? Will the problems of the Middle East spill northwards, through Iran? How will withdrawn-again China react? Where does Japan stand in this changing world? The simple answer is that nobody can tell, which is one of the reasons why present circumstances are cheerful. But that is also a good reason why the "institutionalization" of the safer world cannot be left to the United States and the Soviet Union alone. What Baker needs now, is a political agreement with Western Europe, Japan and the Soviet Union on the future exercise of their political and economic influence. Horse-trading on technology transfer is bound to be an important ingredient, mere money another. □

Diplomacy please

The US human genome project must be mounted internationally, and others must help pay for it.

THE project to sequence the human genome is running into avoidable heavy weather. That is one conclusion drawn from reports last week of the meeting between Dr James Watson, the director of the project for the National Institutes of Health (NIH), and the House of Representatives subcommittee (see *Nature* **341**, 679; 26 October 1989). One teasing issue is that of proprietary rights to whatever sequence emerges. Watson seems not greatly to have exerted himself in disabusing congressmen of the fear that spending $3,000 million of US taxpayers' money would be a gift to the world's biotechnology industry.

In principle, this is a serious issue. Given a complete map of the human genome, commercial companies could scan it for interesting genes or arrangements of genes. Given the possibility that, 15 years from now, much more will be known of how the products of one gene regulate another, a complete sequence may yield even more subtle information about human pathology, and about the usefulness of drugs, synthetic or otherwise. But that is not

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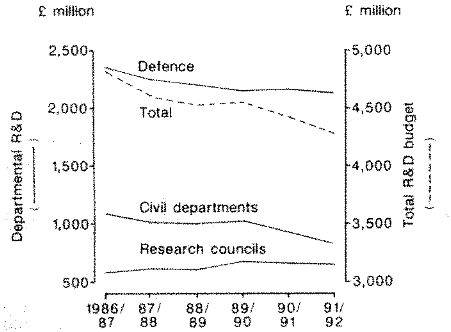
The report, *The 1989 Annual Review of Government Funded Research and Development*, predicts that total government expenditure, in cash terms, will fall from £4,616 million in 1987–88 to £4,282 million in 1991–92. In real terms, using the report's GDP (Gross Domestic Product) deflator, this represents a fall of 30 per cent in the government's contribution. The total expenditure figure is split into three blocks: the funds allocated by the Ministry of Defence, other ministries, and the five research councils and the Universities Funding Council (previously the University Grants Committee).



Research and development expenditure by departments, measured in real terms.

The government last year announced plans to switch funds from "near-market" research to basic research. This shift is reflected in the slashing of funds to the ministries. For example, between 1987–88 and 1991–92, the Ministry of Agriculture, Fisheries and Food's research budget will fall from £113.8 million to £93 million, while the Department of Trade and Industry's expenditure will fall from £324 million to £201 million over the same period.

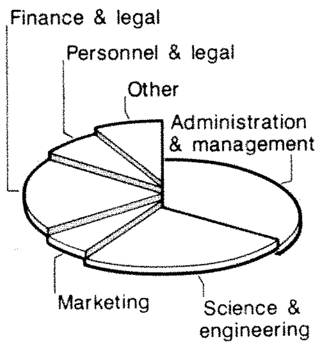
The money saved by cuts in applied research is to be ploughed back into basic research in line with the advice of the Advisory Board for the Research Councils (ABRC) to the Secretary of State for Education and Science. In real terms, the sums available are £13.3 million in 1990–91 and £27.7 million in 1991–92.

There is also to be a transfer of funds from the Universities Funding Council to the research councils. Thus the government's estimate of universities' spending on research out of their general subvention, was £760 million last year, but will fall to £668 million in 1991–92. The overall effect is that, although what is called the Science Budget — the funds spent by the five research councils — will have increased, the total amount of government spending on science will have declined.

Another section of the report shows that Britain is still lagging behind competitors in the proportion of GDP spent on research and development. In 1987, government spending on civil research and development amounted to 0.58 per cent of GDP, compared with 0.91 per cent in France and 0.96 per cent in West Germany. On defence, Britain spent 0.54 per cent whereas the United States spent 0.88 per cent.

The figures also show that, although Britain is getting richer, smaller proportions of GDP are being allocated to research and development each year. Between 1982 and 1987, government spending on civil research and development declined from 0.70 per cent of GDP to only 0.58 per cent. Over the same period, the figure for France rose from 0.84 per cent to 0.91 per cent.

The decline in government support for research has been partly offset by increases in research support from other sources, notably spending by British industry and the activities of international research agencies, especially the European Commission. (The report says that British applicants routinely win more than 20 per cent of the total sums awarded under European Communities' contracts, larger than Britain's 18.9 per cent contribution to the total cost of the Commission's programme.)



First destination of UK science graduates in 1987.

On this basis, the report says that the total value of research and development in Britain financed by all sources amounted to 2.3 per cent of GDP in 1987, the same proportion as four years earlier. During the same period, the corresponding proportions in West Germany increased from 2.5 per cent to 2.8 per cent and, in France, from 2.1 per cent to 2.3 per cent. In Italy over the same period, research spending increased from 1.0 to 1.3 per cent of GDP.

The fall in British government support for research and development is matched by a decline in graduate interest in science careers. The report notes that many science graduates are drifting away from careers in research, preferring service industries. More of the 11,835 science graduates in 1987 chose a job in administration and management than in any other field, including science and engineering.

Only 3,037 graduates chose to stay in research, whereas 4,010 opted for administration and management and 2,125 for legal and financial professions. The popularity of careers in science and engineering among engineers was significantly higher, with 6,412 out of 8,911 graduates staying in the profession.

Ben Webb

AIDS RESEARCH

Have grant, will travel

Bonn

In an attempt to bring some of its best young biologists into AIDS research, West Germany has announced a lucrative fellowship programme. Beginning in 1990, as many as 50 candidates a year will be awarded travel grants and salary (but no automatic research support) to spend two years in a foreign laboratory. Three more years of support will follow after the candidate returns to West Germany.

The programme, which will initially last five years, will be administered by the German Cancer Research Centre (DKFZ) in Heidelberg, with the funds being provided by the West German Research Ministry (BMFT). About DM40 million ($21 million) has been set aside.

Despite the policy of Research Minister Heinz Riesenhuber that no good project should fail for lack of support, the number of suitable applicants for money so far made available for work on AIDS has not fulfilled expectations. Policy-makers are aware that there are structural reasons for the gap (see *Nature* 332, 4; 1989) but are wary of setting up a research centre for AIDS for fear that it would not be flexible enough if research priorities should change.

By reaching out to the younger generation — applicants must be no older than 35 and must have completed a doctorate less than five years before applying — the Research Ministry is trying to encourage future research groups.

Steven Dickman

DIABETES DEATHS

Officials try to allay fears

Washington

HEALTH agencies in the United States and Europe are trying hard to put to rest fears of a connection between the use of human insulin and the deaths of several insulin-dependent diabetics.

The issue, which has caused some alarm in British medical circles in recent weeks, is based upon an apparent increase in the number of deaths, due to "hypoglycaemic unawareness", in diabetics who have switched from animal-derived to human insulin.

Hypoglycaemia, or insulin reaction, strikes diabetics when the level of insulin in their blood is too high relative to their blood sugar level. The hypoglycaemic reaction is normally preceded by numerous physical symptoms such as hunger, sweating and poor coordination, and is reversed by consuming sugar. But anecdotal reports in Britain have suggested that in some diabetics who have switched from animal-derived to human insulin, the warning symptoms of a hypoglycaemic reaction are much less noticeable. This phenomenon has been linked to 17 deaths in Britain in the past year.

No scientifically established link exists between hypoglycaemic unawareness and use of human insulin, but enough concern has already been generated in Britain to have spurred further investigation. As in the United States, most of Britain's 200,000 diabetics have now switched from animal-derived to human insulin. Although some human insulin is manufactured by conversion from animal sources, much of it is synthesized using recombinant DNA technology, a fact that may well have inflamed the fears; human insulin is one of only a few genetically engineered products in wide use.

The UK Committee on the Safety of Medicines announced last month that with the help of the British Diabetic Association (BDA) it would investigate the reports of hypoglycaemic unawareness. In a recent UK survey, 6 per cent of 302 randomly selected diabetics reported diminished awareness of hypoglycaemia after switching from animal to human insulin. An earlier Swiss study, from 1987, reported diminished awareness in more than one-third of the 176 patients interviewed. Meanwhile, the BDA is said to have received some 60 unsolicited letters from patients complaining of hypoglycaemia with human insulin, sometimes with altered awareness.

With concern spreading overseas, the American Diabetes Association last week issued a statement urging people with diabetes "not to overreact". "There are no scientific studies confirming these initial reports", the statement stresses, adding that, given the complexity of mechanisms that warn the body of hypoglycaemia, it "is very unlikely to be due simply to the type of insulin used." Dr Robert E. Silverman, chief of the diabetes programme branch of the US National Institutes of Health (NIH), says that hypoglycaemic unawareness does not seem to be a problem in the United States. Silverman's group is sponsoring a comprehensive long-term clinical study of diabetes, involving some 1,400 diabetic subjects, and so far an increase in hypoglycaemic unawareness "hasn't jumped out of our data", although the issue is not a specific focus of the study.

Silverman is confident that if hypoglycaemic unawareness were a problem among US diabetics, indications would have shown up through the NIH study or in the adverse drug reaction programme sponsored by the US Food and Drug Administration. Although he does not dispute the UK findings, Silverman has reservations about them because they are based upon "retrospective reporting by patients of something that happened in the past". But he says that animal-derived and human insulin are known to have different kinetics of absorption into the bloodstream, and that close monitoring is merited when patients alter their treatment regimens.

Edward West, a spokesperson for Eli Lilly and Co., the largest manufacturer and distributor of human insulin in the United States, warns against what he calls "a great deal of misinterpretation" over the issue, and adds that none of the reported deaths have occurred in patients who were taking insulin manufactured by his company. Nevertheless, West says that his company already incorporated warnings about the potential for altered awareness of hypoglycaemia in its patient literature on human insulin. In response to a request by the British Licensing Authority, all insulin manufacturers in Britain are said to have made the change in their literature as well. **Seth Shulman**

WEATHER FORECASTING

Monsoon prediction model

New Delhi

ACCURATE predictions of the behaviour of the Indian monsoon for the past two years have bestowed a new confidence on the scientists of the Indian Meteorological Department (IMD) and their new long-range forecasting model. Monsoon forecasts have great economic and even political value; the amount of rain that falls from June to September affects not only the agricultural success of large parts of the country but also, as a consequence, their political stability. Earlier efforts to predict monsoon behaviour have, however, been largely fruitless.

The IMD now claims it has a qualitative model good enough to be a useful tool in decision-making. Meterologists say they can predict, two months in advance, not only whether the monsoon will be good or bad but whether total rainfall will be more or less than the normal 88 centimetres. The model is a simplistic one, based on isolated and empirical discoveries of links between a number of regional and global meteorological and oceanic parameters and the monsoon circulation. Fifteen such parameters have been identified as having a historical relationship with monsoon rainfall. There seems to be a link, for example, between the strength of the El Niño phenomenon off the South American coast and the monsoon rainfall in the following year. And extensive Himalayan snow cover in winter, which increases albedo and leads to less continental heating, betokens a poor monsoon that summer.

From a study of monsoon rainfall between 1951 and 1987, IMD meteorologists argue that India has a normal monsoon whenever at least nine of the 15 parameters are favourable. By contrast, 1965 was a year of extreme drought and none of the parameters was favourable that year, whereas in 1955, which saw the heaviest monsoon of this century, only one parameter was unfavourable.

Despite the empirical nature of this model, Vasant Gowariker, secretary to the Department of Science and Technology, says IMD is "quite convinced" that a qualitative prediction of the monsoon can be made simply on the basis of how many of the 15 parameters are favourable. The model was first put to use in 1988, when 13 of the 15 parameters indicated a good monsoon. In May that year, a month before the onset of the monsoon, IMD predicted a monsoon with total rainfall "on the positive side of normal"; the rainfall turned out to be 19 per cent above normal. The following year IMD predicted, this time two months in advance, another good monsoon and, at 30 September 1989, the total rainfall stood at 1.4 per cent above normal. Correct forecasts for two successive years, claims Gowariker, have "validated the utility of our model as a decision making tool". IMD scientists hope to derive quantitative predictions of the monsoon rainfall by weighting the 15 parameters differently. Like any statistical technique, Gowariker says, these models have limitations, "but they are the best available to make long-range forecasts of the monsoon."

K.S. Jayaraman

NUCLEAR CLEAN-UP

Cynics still unsatisfied

Washington

THE US Department of Energy (DoE), under the leadership of new Energy Secretary James D. Watkins, has attracted public attention and a good deal of praise for its announced determination to clean up the nation's contaminated nuclear-weapons production facilities. But even before it has properly begun, DoE's much publicized five-year environmental plan has come under attack, from private organizations and other government agencies, for being over-ambitious, impractical and even misleading.

Senate hearings on the clean-up plan are not expected until later this month and the period for public comment has not ended, but there is already controversy. Officials from the National Academy of Sciences (NAS) and the Environmental Protection Agency (EPA), as well as a variety of independent technical experts, have set out harsh criticisms of the voluminous plan, which was announced in August and unveiled officially in September (see *Nature* **340**, 416; 1989).

Particularly pointed are comments from the privately funded Natural Resources Defense Council (NRDC), which claims that DoE's new accounting procedures exaggerate the true amount allocated to the environmental clean-up. The group claims that the advertised $19,000-million cost of the five-year plan includes basic operating and maintenance functions at the weapons-production facilities, costs which DoE is obliged to spend anyway and which do not represent any new commitment of money to clean-up operations.

According to Jim Werner of NRDC, the DoE's plan includes routine maintenance items, such as replacing waterline valves, in its budget. Numerous similar examples, he says, argue that a large portion of the plan's budget has "nothing remotely to do with environmental restoration". Branding the situation an "old accounting trick", Werner says that his analysis shows that as much as 60 per cent of the DoE plan's financing is merely money shifted from existing budget categories.

Meanwhile, other agencies have criticised the plan's lack of detail. The NAS Board on Radioactive Waste Management, for example, criticizes DoE for not ordering the contaminated sites according to some sort of priority system. Without such a ranking system, the NAS's review declares, the plan lacks "focused information on the specific, near-term actions that DoE plans to initiate" and risks "promising more than can be delivered".

Senior EPA officials have also objected formally that the plan "lacks specificity and depth". In addition, these officials complain that the DoE has not placed a high enough priority on compliance with current environmental laws in the work that it is doing at present at its contaminated facilities. And finally, the DoE has received sharp criticism for not allocating specific funds for research and development activities despite their stated emphasis on an "aggressive R&D program" devoted to the development of "environmental restoration, treatment, and disposal technologies".

Criticism of the DoE clean-up plan comes despite the overall high marks given to the Energy Secretary by most observers. Since he assumed command at the Energy Department, Watkins has been a highly visible and unstinting advocate of everything from increased science education to keeping alive the Shoreham nuclear power plant in Long Island, New York. Notably, Watkins is given credit even by his critics for his repeated and forceful public assurances that the issues of hazardous and radioactive contamination at the DoE's environmentally plagued facilities will henceforth receive the agency's highest priority.

The NAS report, for example, praises what it terms the "much publicised 'change of culture'" at DoE; this refers to an early speech by Watkins in which he admitted that DoE administrators had in the past tended to evade their responsibilities and to let environmental concerns go unanswered.

Many other commentators similarly endorse Watkins' actions, but some are more cynical. James Beard, of the Washington-based Environmental Policy Institute, for instance, acknowledges that there is a new attitude at DoE, but says it is, so far, "more a change in public relations than policy".

Defending the clean-up plan, Watkins has said that he intends it to be a "living document" subject to periodic review and amendment. This sentiment is reiterated by DoE officials, who claim that the plan must be viewed only as a broad "roadmap" for the first stages of a clean-up programme that DoE believes will take 30 years.

Nevertheless, the extent of the current controversy indicates a widespread scepticism about DoE's management record, as well as concern over the enormous federal resources that will be needed to clean up the 40-years' worth of contamination. One influential congressional staff member underlined the crucial nature of the current debate. "The last thing anyone wants", he says, "is to participate in hearings several years from now about how DoE wasted billions of tax dollars in a flawed clean-up program".

Seth Shulman

ALASKAN OIL SPILL

Suit and countersuit for Alaska and Exxon

San Francisco

RELATIONS between the state of Alaska and the Exxon Corporation, already strained in the aftermath of the oil spill last March in Prince William Sound (see *Nature* 341, 174; 1989) reached a new level of tension last week when the oil company announced a lawsuit against the state, claiming that Alaskan officials had interfered with the company's clean-up efforts.

The lawsuit, filed in the Superior Court of Alaska and seeking unspecified damages, drew an angry response. "They're just trying to rewrite history for legal reasons", asserted Ernest Piper, special assistant to the governor. He said the claim was "so contrary to fact that nobody even considered it an issue".

Exxon's complaint is actually a counterclaim to a suit already brought against the company by Alaska, which is seeking unspecified punitive and compensatory damages for alleged violations of state, common and general maritime laws.

The counterclaim focuses on an oil-spill contingency plan approved by the state for what is known as Zone 1, the central portion of the sound where most of the 11 million gallons of crude oil were spilled from the Exxon tanker *Valdez* when it struck a reef.

Both sides agree that the plan allowed chemical dispersants to be used at the discretion of the US Coast Guard alone, without prior approval from the state. But Exxon claims that the state nevertheless made known its opposition to the chemicals, which break down oil into microscopic droplets that are easily dispersed by normal tide motions, to the extent that the Coast Guard delayed granting permission for their use.

As a result, says Exxon, most of the oil remained untreated in Zone 1 for two days. By the time approval came, a violent storm had blown the slick out of the area. "It was a foreseeable consequence of the state's preventing the timely use of dispersants that much more oil would wash onto beaches, shorelines and islands and into intertidal and estuarine areas", Exxon said in the suit, which seeks reimbursement from Alaska for the part of the clean-up costs and damage claims caused by the state's alleged actions.

Piper responded that the state did not interfere at all in the clean-up, and that the Coast Guard delayed its approval solely because of unsuitable weather conditions. He said that dispersants work only in agitated seas, and that for the two days in question the seas were so flat and calm it would have been pointless to use them. "The state in no way delayed any decision to use dispersants", he said.

Robert Buderi

INTERNATIONAL COOPERATION

UNESCO on trial in Paris

Paris

THE twenty-fifth general conference of UNESCO (the United Nations Educational, Scientific and Cultural Organisation), now in progress, will be a testing occasion for the organization and for its director-general, Federico Mayor.

The agenda for the month-long (17 October – 16 November) meeting at the Paris headquarters includes Mayor's medium-term plan for 1990–95 and the budget for the next two years. But also in the balance are his efforts over the past two years to carry out reforms designed to persuade the United States, Great Britain and Singapore to rejoin the organization; they left in December 1984 professing unhappiness with UNESCO's effectiveness and political direction.

Since his election two years ago (see *Nature* **329**, 659; 1987), Mayor's liberal style has, for many, been a welcome contrast with that of his autocratic predecessor, Amadou Mahtar M'Bow. He has also proved himself an able diplomat by persuading the Palestine Liberation Organisation to postpone its demand for membership until the next general conference. Without this withdrawal, there would have been no hope that the United States would reconsider its membership.

But within UNESCO, Mayor has been criticized for not going far enough or fast enough in his reforms. And, despite his three meetings with the new US Assistant Secretary of State for International Organisation Affairs, John Bolton, a Congress hearing on UNESCO in September, at which non-governmental organizations and the private sector were invited to testify, made it apparent that Mayor has still not convinced some sectors of the US administration.

At the hearing, Frank Press, former director of the National Science Foundation, Leonard Sussman, author of a recent book on press freedom and Dr Mitchell of the American Council on Education gave testimony on UNESCO which, according to an observer at the hearing, emphasized its potential future role in international cooperation in science. They implied that the United States could no longer afford to stay outside the organization despite its faults. But Bolton, a Bush appointee who came from the hard-nosed Heritage Foundation, maintained that UNESCO still has a long way to go before reforms justify a US return.

It has been difficult for Mayor to achieve real reforms on the questions that were instrumental in the US and British decisions to leave. At the heart of the quarrel was the New World Information and Communications Order (NWICO), M'Bow's attempt to protect developing countries from what was perceived as an unwelcome flood of media attention from industrialized countries. As part of its programme, NWICO effectively endorsed the right of governments to censor critical news reporting.

In his new medium-term plan, Mayor addresses this issue by falling back on the UNESCO constitution, which speaks of the organization's duty to advance the "mutual knowledge and understanding of peoples" by promoting the "free flow of ideas by word and image".

But Africa is UNESCO's main province of concern, and Mayor cannot afford to upset the African delegations; he thus refers to a need for the "balanced dissemination of information", a locution that to sensitive US ears sounds like an echo of NWICO. The way out of this quarrel, says Mayor, is through the notion that the interests of developing countries can best be served by encouraging the development of endogenous communications and media.

Another cause of US irritation was the top-heavy bureaucracy. Deprived of more than a quarter of its regular income by the US walk-out, the organization was forced to cut its staff of 2,800 by 700. But, as Mayor pointed out in his opening address on 16 October, cost-cutting is incompatible with the injection of much-needed new blood. The inability to hire new staff in any quantity means that Mayor has not only had to manage a programme set up by his unpopular predecessor, but has had to do so largely with M'Bow's former staff.

The content of Mayor's medium-term plan has so far met with approval. His "priority of priorities" is to reduce the "unacceptable gap" between the richest and poorest peoples through education programmes. Next year will be International Literacy Year and UNESCO will hold a conference on Education for All in Thailand in March. Other priorities are the promotion of peace through increased cultural exchange and also of "peace with nature" through UNESCO basic science programmes, a plan whose theme is "not to mortgage the very existence of future generations".

But despite Mayor's ambition that UNESCO should "do less, but in greater depth", the organization is faced with severe financial difficulties. Taking account of a shortfall carried over from the last budgetary period, the proposed $372.5 million regular budget for 1990–91 is effectively a decrease in real terms, and is on a level with spending in the early 1970s. This means that the programme for the next two years will be hotly contested.

Developing countries have already resisted a proposed 2.5 per cent increase in members' contributions, and plans to modernize communications equipment are likely to be postponed.

According to John Fobes, US observer at the conference on behalf of the International Social Science Council, the time is right for the United States to return. Now that the communications issue has quietened down, he says, the value of UNESCO as coordinator of international science, technology and education programmes can be appreciated.

"There will be a lot of important conferences on global issues in the next few years and it would be good if UNESCO became a quiet place for an overall look. In the next ten years, UNESCO will be rebuilt and will become a monitor, instead of a do-all and be-all. The world needs that."

Despite Bolton's luke-warm statement in Congress, some influential groups within the United States are in favour of a return. In Britain, the Foreign Office has assured Mayor that the government will review its position once the conference is over.

Peter Coles

RAINFOREST DESTRUCTION

Unions turn green in Australia

Sydney

CONCERN over the fate of South-East Asian rainforest has led Australian labour unions to take an interest in ecology. The Building Workers Industrial Union (BWIU) for the state of Victoria, noting the daily loss of 11 square kilometres of Malaysian forest, has banned the use of imported rainforest timber. According to the BWIU, more than one-third of such wood is used to build forms for concrete-pouring, and then discarded.

Domestic timber should be used instead, the union believes, which would provide more jobs for Australians, save endangered species of Asian trees and help native groups such as the Penan tribe of Malaysia whose traditional forest habitat is only a year away from destruction.

The BWIU ban is supported by the Australian Council of Trade Unions, which has announced its intention to end the importation of timber from threatened rainforest areas. The Waterside Workers Federation and the Seamen's Union have begun a series of 24-hour actions to prevent the unloading of timber from ships docking in Australian ports.

But according to Neil Goldsmith of the Plywood Association of Australia, their actions could be counter-productive. The import of rainforest timber is a A$128-million-a-year industry, and disruptive union action could, says Goldsmith, drive up prices and cause the loss of domestic jobs.

Tania Ewing

GLOBAL CLIMATE CHANGE

Equivocation on cooperation

Washington

DESPITE President George Bush's election promises to take the lead in tackling global climate change, his administration has outraged the US Congress and environmental groups by hesitating over whether to send official representatives to an international environmental conference in the Netherlands next week. The White House Domestic Policy Council finally agreed that the president's science adviser, Allan Bromley, should accompany Bill Reilly, the head of the Environmental Protection Agency, to the conference, but, it is said, has not allowed Reilly to invite participants to another conference that would be hosted by the United States next year. A new inter-agency working group set up to develop the administration's policy on global climate change will meet this week to decide whether to support the global climate convention that will be proposed at the Netherlands conference.

The controversy hijacked a hearing of the Senate Committee on Commerce, Science, and Transportation last week whose purpose was ostensibly to consider the nominations of James Wyngaarden, the former director of the National Institutes of Health, and Thomas Ratchford to be associate directors of the Office of Science and Technology Policy (OSTP). Bromley denied that the administration was opposed to negotiating a global climate convention, but emphasized that it would undertake no action until the end of 1990, when the Intergovernmental Panel on Climate Change (IPCC) will have completed its studies. Formed under the auspices of the United Nations Environment Programme and the World Meteorological Organisation, the IPCC is reviewing the scientific evidence for global warming, the social and economic impact of possible climate changes and ways of dealing with the problem.

Senator Albert Gore (Democrat, Tennessee) said he was "extremely disappointed" with the advice Bromley was giving to Bush on this issue after Bromley's refusal to say whether he had initially been opposed to attending the conference or whether he was in favour of negotiating a convention were interpreted as indicating his opposition to both. Although earlier this year Bush signed a declaration at the G-7 economic summit that "strongly advocates" common efforts to limit emissions of carbon dioxide and other greenhouse gases and declares that a global convention on climate change is "urgently required", Bromley said that there was a difference of opinion between the administration and Congress over the definition of 'urgent'. He also said that there was "a substantial distance to go yet" before an adequate understanding of the science of global change would be reached, and quoted a recently revised model from the UK Meteorological Office that reduced the predicted temperature increase for a given addition of carbon dioxide from 5.2 to 1.9 °C after a more sophisticated treatment of cloud behaviour was added. Bromley also emphasized that discussion so far has focused too much on "the input side of the global change equation" and that more studies are needed of the economic consequences of a convention.

Eleven environmental groups, including the Natural Resources Defense Council, the Environmental Defense Fund and the National Audubon Society, offered Bush rather different advice last week on policies to tackle global warming. In a joint declaration, they called on Bush to support a 20 per cent reduction in national carbon-dioxide emissions by the year 2000, an international convention to reduce emissions of greenhouse gases, and the development of a national climate policy. At a hearing of the House of Representatives subcommittee on human rights and international organizations, they all endorsed a bill to coordinate better federal research on climate change through the Committee on Earth Sciences of the OSTP. **Christine McGourty**

ENVIRONMENT

'Hot lab' to be closed over contamination fears

San Francisco

BOWING to growing community concern over toxic contamination, and citing changing business needs, the Rocketdyne Division of the Rockwell International Corporation has decided it will shut down a nuclear laboratory, used for processing radioactive materials, at its Santa Susana Field Laboratory east of Los Angeles.

The decision, announced on 20 October and due take effect exactly a year later, will close a 30-year-old operation. Making the announcement, the company said there had been a change in federal government emphasis away from the liquid-metal fast-breeder reactors, in which Rocketdyne had done specialized contract work, towards light-water reactors.

But Rockwell has also been suffering from revelations that a US Department of Energy survey had found radioactive and chemical contamination of the soil beneath the 2,600-acre Santa Susana site, although no immediate health hazard was declared.

Rocketdyne had originally requested a 10-year extension of its operating licence from the Nuclear Regulatory Commission (NRC), but has now asked for an extension only until 20 October 1990 to allow time for the company to complete its decontamination and decommissioning plan for the facility. Robert Buderi

IVORY TRADE

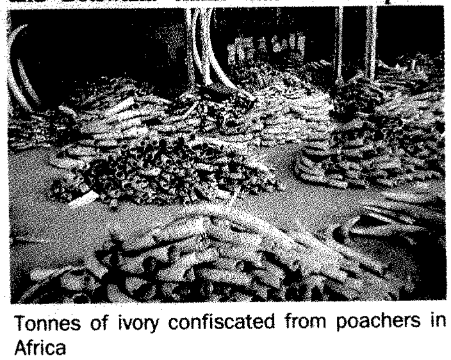
Japan agrees to total ban on imports

Tokyo

JAPAN, the world's largest importer of ivory, has at last agreed to a total ban on ivory imports, at least for the time being.

The Japanese cabinet decide on 20 October to abide by the ban on all imports implemented by the Convention on International Trade in Endangered Species (CITES) at a meeting in Lausanne, Switzerland, earlier in October. Japan at first joined South Africa, Zimbabwe and Botswana in opposing calls for a ban but then abstained at the final vote.

CITES member nations are concerned about rapidly dwindling elephant populations in Africa. But South Africa, Zimbabwe and Botswana claim that their elephant populations are stable or increasing due to conservation measures and wish to continue their lucrative ivory trade.



Tonnes of ivory confiscated from poachers in Africa

In June, the Ministry of International Trade and Industry (MITI) imposed a ban on indirect ivory imports from suppliers in such places as Hong Kong and Singapore. But ivory importers immediately began to stockpile and MITI responded with a 'temporary' total ban in September (*Nature* 341, 270; 1989).

Japan's decision to abide by the CITES ruling, rather than filing a reservation to get around the ban as several African nations plan to do, may in part be due to the fact that Tokyo will host the next CITES meeting in 1991. If imports had continued, Japan would be in a very embarassing position.

But Cecilia Song of TRAFFIC Japan, the trade monitoring wing of the Worldwide Fund for Nature (WWF), says that MITI and the ivory industry hope to resume trade in the near future. The Lausanne agreement allows for resumption of trade after two years if countries can show they have developed an effective strategy to protect elephant populations.

In the meantime, Japan's ivory importers have begun importing mammoth tusks from the Soviet Union. David Swinbanks

BERLIN ACADEMY

Move raises problems

Munich

The uncertain life of the West Berlin Academy of Sciences, which must leave its present home by July next year, seems destined to continue. Criticism from the opposition Social Democrats (SPD) in the *Land* of Hesse may prevent the beleaguered academy from moving there, despite earlier indications that Hesse would be a safe haven. The SPD has set "high hurdles", according to a spokesman, before it will accept the academy.

The academy became a source of discord in Berlin politics after it was pushed through the parliament in 1987 by the conservative Christian Democratic Union (CDU; see *Nature* **329**, 659; 1987). SPD and Green Party politicians, then in the opposition, criticized the academy as a "debating club" with conservative leanings. They vowed to shut the academy once they gained power and, in May this year, the now left-wing government of West Berlin proposed a law to do just that.

With the help of his conservative coalition partners, Hesse science minister Wolfgang Gerhardt (Free Democrat) responded to the Berlin decision in June by offering to "adopt" the academy, and to give it the same financial support as it had been receiving in West Berlin — DM 8.5 million ($4.6 million) a year. This offer needs the approval of the *Land* parliament of Hesse: debate is about to resume, and a final vote is expected in December or January.

But now academy president Horst Albach has said that he will not accept the offer unless the SPD in Hesse also agrees to support the academy. Having already been the victim of a change of government, Albach wants to avoid further uncertainty over the future of the academy.

In response, the SPD in Hesse has demanded a role for political parties in the academy's board of directors as well as strong representation by the universities and research institutes of Hesse. "The academy will be a big financial burden for Hesse", said SPD spokesman Erich Stather. "It is only logical that we want a chance to express our views. What bothers us the most is having the academy floating free of all other scientific institutions." The SPD met in private this week to discuss its position on the academy.

Will the academy be able to accept the terms demanded in Hesse and still remain independent? Academy spokesman Eberhard Vogt calls the problem a case of "Scylla and Charybdis" — the academy is trapped between two hazardous alternatives. Some members of the governing board, which manages the day-to-day affairs of the academy, oppose an industry-financed future for the academy for fear of losing independence.

Nevertheless, the mood in the academy is optimistic. "We have been criticized by conservatives at the University of Frankfurt for being too left-wing, whereas left-wingers in Kassel have said we are too conservative", he said. "So we must be in just the right place politically now." Albach is grateful for the chance to correct the "birth defect" in the way the academy was voted into existence in West Berlin, and he has been crisscrossing Hesse in an attempt to generate support for the new version of the academy.

Ironically, if the academy were to accede to the demands of the SPD in Hesse, it would belatedly live up to the demands that the SPD in West Berlin proposed when its future was discussed in 1986–87. But this is no longer enough for the West Berlin Senate to invite the academy to stay: the Greens would oppose any such move as a violation of their coalition agreement with the SPD.

In a further irony, some activities of the West Berlin academy will probably be allowed to stay in the city if a compromise is reached in Hesse. Hesse is expected to approve the existence of such a "satellite academy" and there is even talk in the West Berlin SPD that some new academy projects would be welcome — a belated tribute to what some are calling the "academy in exile". **Steven Dickman**

BRITISH UNIVERSITIES

United stand in move to greater efficiency

London

Four British universities have appointed a firm of management consultants to try to make them more efficient. In the first move of its kind in higher education, the Universities of Birmingham, Leicester, Loughborough and Warwick have joined forces to maximize their use of resources to offset tighter government budgeting.

The PA Consulting Group will carry out efficiency audits of services such as residences, libraries, maintenance and catering in the four universities.

For the next three years, PA will draw up comparative studies across the four universities and will also look at each area specifically.

Professor Gerald Bernbaum, executive pro-vice-chancellor and registrar of the University of Leicester, said the universities were simply following Universities Funding Council advice to carry out an audit. He said PA would help them "share good practices" and that he was confident the study, which was at a "reasonable cost", would pay for itself in time. Ben Webb

SOUTH AFRICAN EDUCATION

New minister, old problems

Cape Town

In a cabinet reshuffle last month, the new South African president, F.W. de Klerk, announced the return of Dr Gerrit Viljoen as Minister for National Education, with an additional appointment as Minister for Constitutional Development. Viljoen, a graduate of the Universities of Cambridge and Leiden, was professor of Greek at the University of the Witwatersrand in Johannesburg before entering politics, and had previously served as education minister from 1980 to 1984. He was subsequently made Minister of Cooperation and Development, but a year later was effectively moved to the sidelines as Minister of (African) Education and Training, reputedly because he was not a member of ex-President Botha's inner circle. But he was one of de Klerk's staunchest supporters in deposing Botha, and has been rewarded with two of the most crucial portfolios in the new cabinet.

On top of his responsibility for overseeing constitutional reform, Viljoen is faced with the task of defusing the country's growing educational crisis. In 1986, the government declared a commitment to the elimination, over a 10-year period, of racial inequalities in primary and secondary education, but earlier this year was forced to abandon that policy as economically impossible. Education already accounts for 20 per cent of budget spending in South Africa, and in the current economic climate the increases that would be needed for large-scale educational reform are unlikely to materialize. And despite its goal of educational equality, the government is fiercely committed to the provision of separate educational facilities as it regards integration of schooling as an issue too sensitive for the white electorate to accept.

Yet there are signs that this same electorate may be ready to face up to the reality of the situation. The Johannesburg High School for Girls, threatened with closure due to dwindling numbers of white students in its catchment area (which has become a *de facto* mixed area in recent years) decided earlier this year to go private instead. The state has allowed integration in private schools for the past decade, and has also given them limited state subsidies without taking away their charitable status. By allowing a state school to become independent, the government can allow integration without taking overt responsibility.

Academics in South Africa are generally optimistic about Viljoen's appointment, because of his credentials and also because during his first period in the education office universities and museums were well-treated. But he now has more difficult tasks to deal with, and fewer resources to apply to them. Michael Cherry

CHERNOBYL

Lax standards confirmed

London

VIKTOR Bryukhanov, director of the Chernobyl nuclear power station at the time of the 1986 disaster, has at last broken his silence and given an interview to the newspaper *Sotsialisticheskaya Industriya*. Bryukhanov, who is serving a 10-year sentence in a labour colony, had been afraid that any statement he might make would be interpreted as self-justification. "And I was guilty", he admits, "only not as guilty as the court decided." On the night of the ill-conceived experiment that ended in explosion, Bryukhanov was at home in the nearby town of Prypyat.

He was awoken, he says, by a telephone call from the head of the chemical unit who told him only that "something awful has happened — some sort of explosion". Imagining something like a ruptured steam-line, he hurried to the station, but when he saw that the top of number four block was completely missing, "my heart stood still", he says. He telephoned Moscow and tried to order an immediate evacuation, but no one would support him because it was "so firmly fixed in their minds that nothing could happen to the reactor".

Bryukhanov's account confirms the picture which emerged at the post-accident review conference held by the International Atomic Energy Agency of a naive faith in the safety of nuclear power coupled with a neglect of the very rules and procedures on which safety depended. But as director of the Chernobyl power plant from its inception, he is able to reveal the background to this atmosphere of rule-breaking. Starting with a green-field site, he had to oversee the construction not only of the power station itself but also temporary accommodation and later a town for the site-workers. He found himself coping with broken heating systems and failing supplies of fresh vegetables and, unlike the directors of manufacturing plants, he had nothing he could barter to get consumer goods for his workers.

Bryukhanov maintains that without rule-breaking, the Chernobyl station would never have been completed. Electric cables, for example, should have had fire-resistant claddings, but none was available and ordinary cables were used instead. He says he was worried about the situation, but knew that if he made a fuss he would simply be replaced by a new and more amenable director. "It is not within the power of one man to overthrow a system of economic-management relationships which has built up over decades. And he should not be held guilty if in the end he becomes the slave of that system."

But Bryukhanov was found guilty while others in higher places escaped to honourable retirements. Long before the Chernobyl accident, he says, scientists from the Kurchatov Nuclear Energy Institute warned Academician Anatolii Aleksandrov (president of the Academy of Sciences and a leading supporter of nuclear power) of design faults in the RBMK reactor, but nothing was done.

After the accident, the safety systems were tightened up, making it impossible to withdraw many control rods (as was done at Chernobyl) and considerably reducing the automatic shut-down time. Yet Bryukhanov says he is not certain that events similar to those of Chernobyl will not be repeated elsewhere.

Bryukhanov has been heavily criticized since the accident, and the attacks continue. Only last summer, the weekly *Argumenty i Fakty* said that the three operators who carried out the ill-fated experiment on the reactor did not know that pulling out too many rods could cause an explosion. Their superiors did know, the paper said, but they were safely asleep at home.

Whether Bryukhanov did know the full danger and could have stopped the experiment is unclear. He portrays himself as a man in an impossible job, who was made the scapegoat when disaster struck. In the words of Georgii Kopchinskii, head of the Department of Atomic Energy of the Bureau of the Council of Ministers of the USSR, the court "did not judge the man, but the job". **Vera Rich**

CLINICAL RESEARCH

Network of centres better than just one

London

PLANS by the UK Medical Research Council (MRC) to establish a national clinical research centre have been rejected by the Advisory Board for the Research Councils (ABRC), which favours instead a network of provincial research centres. These, says the ABRC, will meet the desired aims of improving the quality of science and the standards of medical training and hospital practice, but will be more cost-effective than the MRC's strategy.

Although Dr David Rees, MRC secretary, welcomed the ABRC's support for the clinical research initiative, he was disappointed that it had not approved the idea of a single centre of excellence. The proposed centre would have been located at Hammersmith Hospital, in association with the Royal Postgraduate Medical School, at an initial estimated cost of £100 million. That figure was later scaled down to £50 million after the ABRC requested that funds be released for other leading clinical research centres in the United Kingdom.

The ABRC recommends that the Hammersmith centre should be fostered, and that the Clinical Research Centre (CRC) at Northwick Park should be closed so that resources can be redirected to the provincial centres. Sir David Phillips, ABRC chairman, in a letter to John MacGregor, Secretary of State for Education and Sciences, says there is a strong case for "enhancing and sustaining" clinical research at the RPMS through an infusion of basic biomedical research, but adds that the ABRC does not favour a single national centre, preferring a "distributed approach" with "phased developments" at a number of provincial centres. Phillips concludes that this programme would cost "significantly less" than the single-centre option.

MacGregor said he was "naturally pleased" that the ABRC's preference on merit was for the cheaper approach. Dr James Scott, of the CRC, said the MRC had "gone for the stars" in asking for backing in the national research centre initiative and that it should not be too disappointed that it had not got complete support. He said the CRC had not been the success it should have been and that clinical research needed to "pull its socks up". Ben Webb

AIDS

AZT for children in extended US trial

Washington

THE US Food and Drug Administration (FDA) last week approved the use of the anti-AIDS drug AZT (zidovudine) to treat children.

Under the terms of a treatment IND (investigational new drug) programme, AZT will be provided free of charge by its manufacturer, Burroughs Wellcome, not only for children showing symptoms of AIDS but also for those lacking symptoms but in the advanced stages of infection with HIV (human immunodeficiency virus).

Two months ago, on the basis of trial results demonstrating that AZT slowed the progress of HIV infection, the drug was made available to asymptomatic but infected adults as well as those with overt AIDS. The new extension is also based on trial results: a study of more than 200 children has shown that AZT can both relieve symptoms and prolong survival. What had held back use of the drug for children was the fear that side-effects could be more severe in children than in adults, but the study results so far indicate that side-effects are not qualitatively different in children. David Lindley

For unto him that hath . . .

Virginia Trimble

The concept of P-selection, by which graduates of the more prestigious (P) universities tend to publish longer than those from other (NP) universities, is herewith introduced into the literature.

If you really want a career in scientific research, then get your PhD from the most prestigious university that will take you. This conclusion (which should surprise very few) follows from data collected for the Astronomy and Astrophysics Survey Committee, now in the process of preparing a report to the US National Academy of Sciences on Astronomy and Astrophysics for the 1990s.

To be precise, astronomers who received their doctoral degrees from a university that ranks repeatedly among the 'top three' American astronomical PhD programmes[1] continue to publish in the field about twice as long as those with degrees from a university that ranks in the 'second ten'. In addition, they are about twice as likely to have current jobs that primarily involve research and/or advanced teaching at observatories or PhD-granting universities.

One can imagine a variety of reasons for the difference. The more prestigious institution may genuinely provide a better education, or it may skim the cream from the available pool of entering students. Or its name may serve as a lubricant to smooth the paths to research grants, acceptance of papers and good jobs for its alumni, at least early in their careers. The data say nothing whatever about the relative importance of these or other causes for the phenomenon, though I am privately of the opinion that the third factor is not negligible.

The sample populations include 106 recipients of PhDs between 1952 and 1988 from the high-ranking institution (hereafter P) and 94 recipients of PhDs between 1966 and 1988 from the lower-ranking one (NP hereafter). Figure 1 shows their publication histories. The horizontal axis is number of years since receipt of doctoral degree. The vertical axis is the fraction of all astronomers who had had their degrees for at least *N* years who were still publishing papers listed in the semi-annual issues of *Astronomy and Astrophysics Abstracts*[2] at least *N* years after their degrees. Upper points pertain to P and lower ones to NP, and the differences are large enough to require no words to guide the eye.

The doctoral programme at P is the older one, and it might be hypothesized that the difference shown in Fig. 1 is a result of good jobs being easier to get in the past (back when our towers were made of real ivory rather than plastic and all our students were above average). As a test of this hypothesis, Fig. 2 shows the publication histories of post-1966 degree recipients from the two universities (85 from P, 94 from NP). Indeed the difference between the two is smaller than in Fig. 1, but the P astronomers are still about twice as likely to continue to publish for more than 18 years (when both samples drop below 10 members) than are the NP ones.

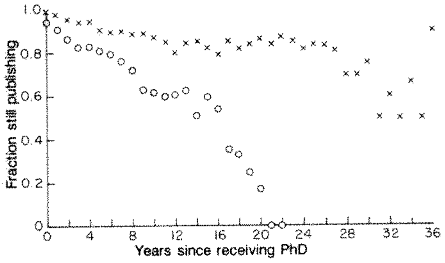


FIG. 1 Fraction of astronomers who have held PhDs for at least *N* years who are still publishing research papers *N* years after their degrees. Upper points = people from the prestigious university, P; lower points = people from the non-prestigious university, NP. Neither set of points starts out a unity because a few people from each place never published anything. Numbers drop below 10 people at 18 years for NP and 28 years for P.

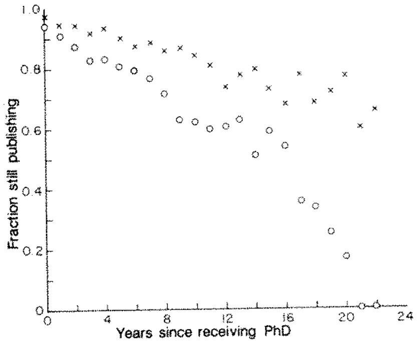


FIG. 2 Fraction of post-1966 degree recipients who are still publishing in astronomy as a function of number of years since the PhD. Upper points = P alumni; lower points = NP alumni. Numbers drop below 10 people between 18 and 19 years post-PhD for both.

Current employment patterns of the two groups are also significantly different. There are members of each group in each of nine categories: (1) astronomy research and teaching at observatories and PhD-granting universities; (2) astronomy research at government and industrial laboratories (like Los Alamos National Laboratory and AT&T Bell Laboratories); (3) research in other sciences; (4) scientific support services (data processing, hardware and software development); (5) industrial employment; (6) government employment (generally science administration); (7) teaching at places with no PhD programme in astronomy; (8) employment outside science; and (9) lost to follow-up and not publishing in astronomy (two from P, three from NP).

The most striking difference is that 65 per cent of the P astronomers, but only 32 per cent of the NP ones, have jobs in category 1. A large majority of incoming graduate students at both places describe their career goals as research and advanced teaching in the field. Most graduate programmes concur that their primary purpose is the production of the next generation of researchers. Thus the P astronomers can be described as more successful than the NP ones by their own standards.

Life being somehow easier in the past is not the primary cause. An artificial sample of P astronomers, age-matched to the NP ones, has 66 per cent of its members in the most desirable category of employment, just the same as for the total P sample. Curiously, this does mean that things are getting worse. People continue to diffuse out of the astronomical research community over their entire careers. But they almost never come back (though there is diffusion into the field of PhDs from physics and other sciences). No one in either sample resumed publishing after a hiatus of as long as five years. And the few returns to jobs in categories 1 and 2 occurred only when the excursions had been brief ones to positions in science administration. Thus we can expect that, 10 or 20 years from now, the fraction of the 1966–88 P astronomers still in research and advanced teaching will be less than 65 per cent and so smaller than the fraction of the total P sample now engaged in these activities.

The moral of the story is thus two-fold: it still pays to go to the most prestigious graduate institution you possibly can, but the gold paving, even on the streets of P, is not as thick as it used to be. □

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1. Gourman, J. *The Gourman Report* (National Education Standards, Los Angeles, 1987).
2. Esser, U. *et al.* (eds) *Astronomy and Astrophysics Abstracts*, Vol. 45 and earlier volumes (Springer, Heidelberg, 1989).

Criminalization of drugs

Sir—Your leading article "Bush declares war on drugs" (*Nature* **341**, 1; 1989) is right in noting that if drug-related crime is to be totally eliminated, all drugs would have to be decriminalized, but it must be hoped that neither the United States, nor any other country for that matter, ends up decriminalizing narcotics or illicit drug use altogether.

You ask "would not a society in which narcotics were accessible that did not destroy itself by addiction be a stronger society and perhaps even kinder and gentler as well?". Problem drug-users have frequently told me, in the ten years in which I have worked with them, that "heroin blanks you out, it makes you emotionally distant". Many other drugs have even nastier and more antisocial effects. In the affluent West, the desire to achieve self-realization is common. Unfortunately, because of large-scale pharmaceutical drug use, it has become a drug-focused society. To be realistic, heroin and other euphoric and mind-altering drugs, whose use can at first be pleasurable, would be used on a wide scale if they were easily accessible. Would we want large sections of the population wandering around emotionally isolated and cut off from meaningful relations with others? Such a society would hardly be "kinder or gentler".

Clearly some degree of criminalization is necessary. But what is needed is a sensible policy suited to the real needs of containing the drug problem. For example, heroin, cocaine and crack are this decade's fashion. Designer drugs will be the major threat in the future. Should there not be international laws outlawing and controlling the illicit manufacturing of all synthetic drugs before they become a fashion?

Further, the British did not, as the author claimed, give up prescribing narcotics some 20 years ago because of the over-prescribing of one doctor. Rather, in the 15 years after the drug dependency clinics were set up in 1968, their consultant psychiatrist directors generally found that such long-term prescribing was medically harmful. Also, it generally did not help addicts to lead normal lives or keep them from crime. Even so, a small number of doctors continued to maintain addicts. Recently, in a move to combat AIDS, their numbers have increased. Most, however, offer only short-term treatment with reducing doses and aim to wean their patients off drugs as soon as possible.

Liberal prescribers such as Trebach argue that the British system of treatment was the reason why, until the 1970s, Britain had only a small drug problem. But, as I have indicated elsewhere, it was precisely because numbers were small that such a policy could be pursued. When the size of the addict population rapidly escalated in the 1980s, treatment and prescribing policies were ineffective in controlling the size of the addict population because heroin was cheap and easily available on the street. While illicit drugs remain easily available on the street, many of the addict population will, for a number of reasons, prefer not to seek maintenance prescriptions, but seek to buy illicitly — if for example, they are only recreational users, or they hate authority and the restrictions imposed by drug dependency units or prefer the excitement of the illegal subculture. And the drug market is organized in so complex a way that it would stimulate a demand on the street for their pharmaceutical drugs. Instead, large-scale liberal prescribing would increase the size of the illicit market, for it would lead to an extensive pharmaceutical blackmarket fed from the overspill from liberal prescribing.

It would be a very foolish policy-maker indeed nowadays who confused 'treatment' with drug control policy or containment.

Angela Burr

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Collision course

Sir—The race between the Stanford Linear Collider and the Large Electron–Positron Collider at CERN, the high-energy physics laboratory near Geneva, to be the first to elucidate the properties of the Z^0 particle raises fundamental questions about the purpose of particular scientific research projects in an era of increasing costs and declining resources. It was known from the beginning that the CERN machine would be able to produce Z^0s in much larger numbers than the Stanford machine. Thus, there was never an expectation that Stanford could produce data that would not eventually be available from CERN. The only question was whether Stanford could produce some of these data first. If some properties of the Z^0 had become known a few months earlier because of the Stanford experiment, it would have had absolutely no effect on scientific progress. It would, however, have been of significant advantage to the careers and reputations of the scientists involved in the Stanford experiment.

When the cost of a single machine such as the Superconducting Super Collider can approach the gross national product of a small developing country, we should question whether the taxpayers should be continuously called upon to support this kind of pointless competition and duplication of effort. If CERN, the United States, the Soviet Union and Japan cannot get together to fund and build a single high-energy physics machine, we should at least try to ensure that expensive facilities in one country complement rather than duplicate the capabilities of machines available elsewhere.

Robert J. Yaes

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Stanford scandal

Sir—Dr Donald Kennedy (*Nature* **341**, 180; 1989) was not president of Stanford University at the time of the assault on Professor Leonard Hayflick, to which I alluded in a review — he was, I believe, head of the Food and Drug Administration in Washington — but the affair caused an international scandal, and unawareness among faculty members, even those who were not physically present, implies the lack of academic solidarity of which I complained.

I think that if Kennedy reviews the history of this affair, he will find my comments neither unjust nor intemperate.

Alex Comfort

The Windmill House, The Hill,
Cranbrook, Kent TN17 3AH, UK

Six of one . . .

Sir—Although one instinctively feels that all information is of value, the data quoted in your recent News and Views comment (*Nature* **341**, 181; 1989) on the British National Survey of Sexual Attitudes and Lifestyles suggest that Mrs Thatcher was right to doubt its value.

You quote the results of a preliminary survey as showing the average (lifetime) number of heterosexual partners as 11.0 for men and 2.9 for women. These figures are literally impossible. A heterosexual union is analagous to a heteronuclear chemical bond, and the total number must be the same if viewed from the male or female end. Thus the average numbers of partners must obey a 'bond consistency' condition

$$c_M N_{MF} = c_F N_{FM}$$

where c is a concentration and N_{MF} the number of female partners per male. Because by empirical observation the concentrations of males and females in the United Kingdom are the same, so must the average number of partners be. The survey results are clearly biased: probably both sexes bias their replies ("lie") in socially acceptable directions. The sociologist you quote was clearly right to be sceptical.

S. J. Gurman

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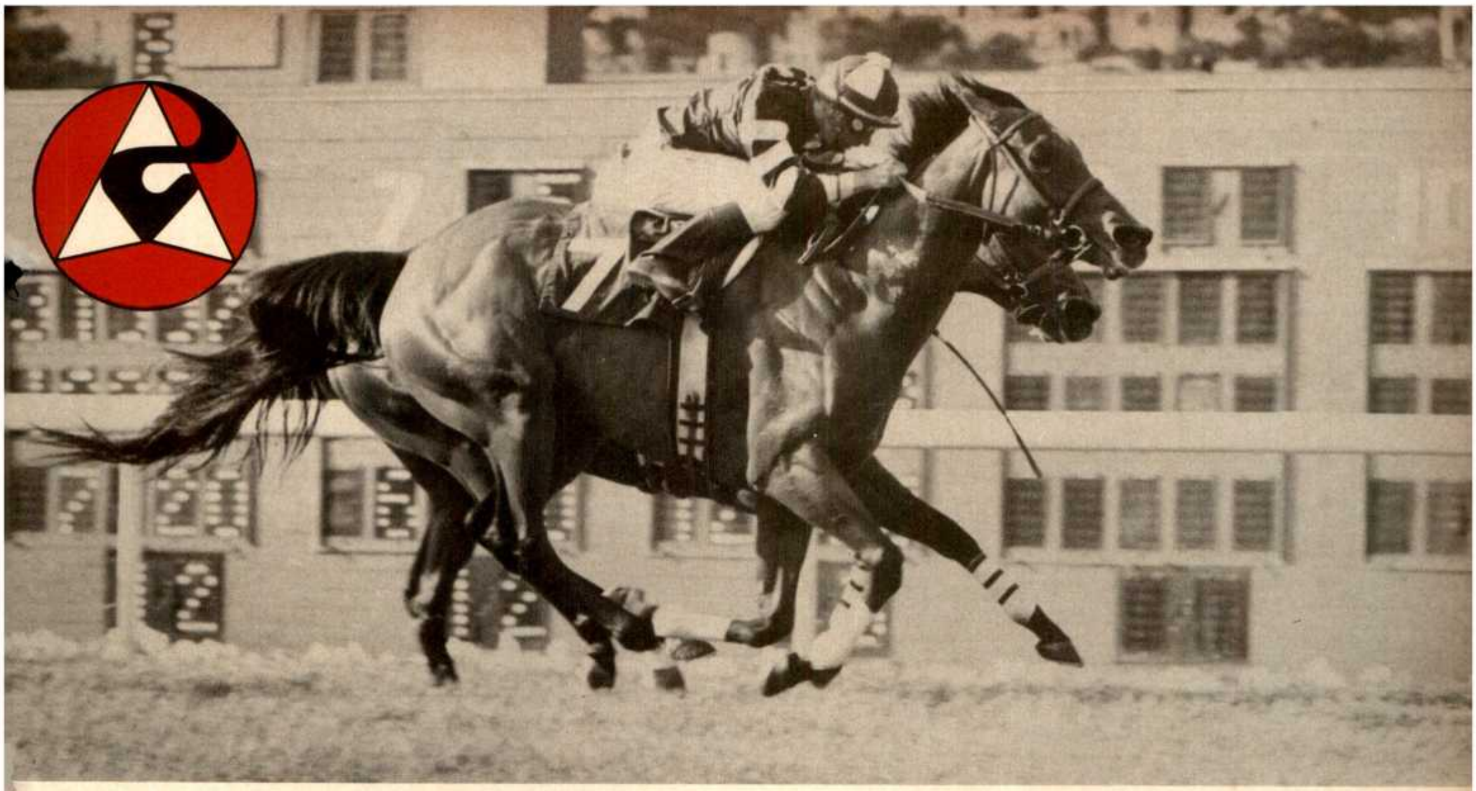

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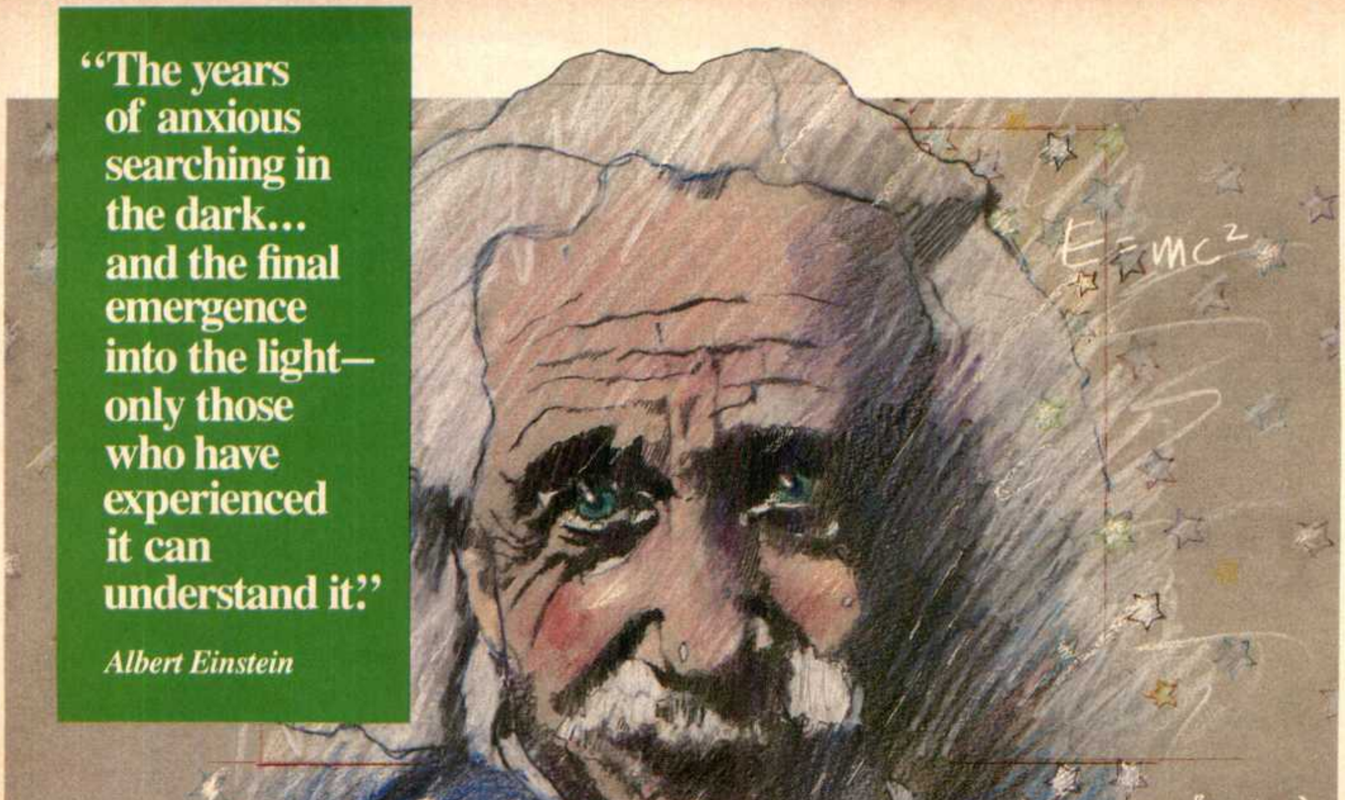

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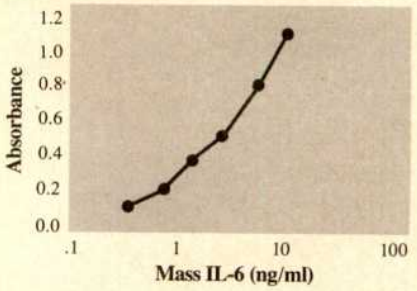

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Mechanical energy from light

In principle, it should be possible to turn radiation directly into mechanical energy, but the best designs so far of machines for doing this consist of paper studies. Somebody should build one.

LIKE mixing chalk and cheese, it grates on expectations of what the natural world is like to be asked to convert light energy directly into mechanical energy, to use sunlight for driving the transmission of a motor-car, for example. Despite present interest in what are called 'renewables', ecospeak for sources of energy alternatives to fossil fuels and uranium, the question is hardly ever asked. And if it were, the answer would almost certainly be a reference to well-tried space technology: make electricity first, and use that to drive the machine.

But that is too cowardly an answer. There is a direct conversion route, even a way of making light drive a piston along a cylinder, as in an internal combustion engine. What can that be? At this point, one must bury the thought that the explanation has to do with the Poynting effect, or with the well-attested truth that photons carry momentum and thus exert a force on whatever absorbs them. Toy radiometers are familiar enough; their aluminium sails do indeed spin round when exposed to light, but few would expect them to do much more — drive a motor-car, for example.

The true design of a light engine proceeds differently, beginning with a working fluid capable of absorbing light and behaving differently as a consequence. Suppose, for example, that one chooses a coloured gas, say a halogen vapour, and exposes that to sunlight. Energy will be absorbed and converted into heat, so that the pressure will increase. In a suitable arrangement, in a cylinder fitted with a piston for example, a modicum of work could be extracted. But the rate at which energy could be extracted from such a device — technically, its power — would necessarily be limited by the speed with which the working fluid could be restored to its condition at the beginnning of the cycle, and thus by the rate at which energy is lost by heat transfer to the surrounding medium. Nobody would expect even that to be enough to drive a motor-car.

Is there some way of augmenting that effect? This is the goal at which the designers of the light engine have set their caps. This is how the argument might go (as rehearsed as early as 1973 by A. Nitzan and J. Ross, *J. Chem. Phys.* **59**, 241; 1973). The trick is to choose as a working fluid a chemical species which is an efficient absorber of radiation, but which is itself replenished whenever its effectiveness as an absorber has been neutralized by having absorbed a photon, so that it is in an excited state.

Specifically, call the absorbing species A and the excited species A*. Suppose that A is itself a dissociation product of another chemical species B, that equilibrium is reasonably well satisfied between the species under the conditions in which the light engine functions and that the reaction by which B is converted into its components (including A) is endothermic. That would mean that, if the temperature increased, there would be more of the product (A*) in the absorbing gas, which would imply positive feedback. One could hope to keep the process of light-absorption going not for milliseconds but for tenths of a second or thereabouts.

That is the point at which three members of the department of chemistry at the University of Chicago get down to business. S. J. Watowich, K. H. Hoffman and R. S. Berry (*Il Nuovo Cimento* **104B**, 131; 1989) choose as their working fluid the fluorosulphate free radical — stoichiometrically SO_3F, which is nothing but SO_4 in which one O has been replaced by an F. But SO_3F has the interesting property of forming dimers that are neither free radicals nor absorbers of radiation; $S_2O_6F_2$ is simply a reservoir from which absorbing gas can be regenerated whenever there is a tendency for the temperature to increase.

In simple terms, the consequence is chiefly to increase the duty cycle of a light engine based on a light-absorbing molecular gas. But the increase may in principle be very great. If A* is not fluorescent, energy from the excited molecule can escape only by means of molecular vibrational modes, a process of thermal degradation which both restores the original absorbing molecule and which must separately increase the concentration of the absorbing species by shifting the chemical equilibrium.

How to make a light-driven mechanical engine from those ingredients? Watowich *et al.* do their best. Implicitly, they acknowledge that, in the construction of a novel energy-transducer, what matters is not theoretical efficiency but power output per unit of mechanical engineering effort. For that reason, their design study ends up with SO_3F embedded in more than 1,000 times as much buffer gas, perhaps molecular nitrogen (to increase heat conduction and thus reduce the length of the duty cycle). A large part of the trick is to operate the light engine in a condition in which the working fluid is metastable — endowed with a greater potential than would be found at equilibrium to replenish stocks of SO_3 depleted by the absorption of radiation.

Several difficulties underlie their calculation, at least one of which is philosophical. Ehrenfest's law of detailed balancing implies that, if one can define a molecular species of any kind, one can, in a steady state, equate the total rate at which it is created with that at which it is destroyed. But Ehrenfest was innocent of quantum mechanics, and would not have worried much about the possibility that a dimer in equilibrium with its monomer in the presence of excited versions of the latter might not behave as if the old-fashioned law of mass action were universally applicable. Ordinarily, of course, he would have been on sure ground.

But the mechanical light-engine requires that the conversion of dimer into monomer molecules should be so much more rapid than the rate at which excited monomers lose energy through conversion to intramolecular vibration, and thus enhanced collision frequency, that excited and non-excited monomer states are never confounded. That is probably a mistaken supposition in circumstances in which the effectiveness of the light engine depends on the speed at which the duty cycle can be completed.

These are strictly theoretical objections, of course. They could be answered simply and conclusively by a demonstration that it is possible to build a machine, with some working fluid or other, that will drive a flywheel when it is exposed to a light, perhaps one more intense than sunlight. Sadly, this seems not to have been done. In the tradition of *Il Nuovo Cimento*, Watowich *et al.* hew to the principle that a gram of theory counts for a tonne of experiment, and is usually easier. For what it is worth, the paper efficiency of a working machine turns out to exceed 0.25, just a little greater than that of the best amorphous silicon converters of solar energy into electricity. But it would be more than just fun if somebody were to build one, even a much less efficient version. Then, at least, there would be a tangible incentive to improve on paper performance.

John Maddox

CELL BIOLOGY

The cell cycle as a *cdc2* cycle

Andrew W. Murray

ORDERLY cell division depends on the coordination of the events of the cell cycle. In eukaryotes this coordination is produced by the regulation of three cell cycle transitions: entry into mitosis, exit from mitosis, and passage through a point in G1 named Start, that commits the cell to initiating DNA synthesis (the S phase). It is now believed that in all eukaryotes each of these transitions is regulated by controlling the activity of a single protein, pp34 — the product of the *cdc2* gene. A model of the cell cycle as a series of closely regulated changes in the activity of pp34 is shown in the figure. On page 39 of this issue[1] Gould and Nurse show that one of these changes is the dephosphorylation of a single tyrosine residue on pp34 and that this reaction facilitates entry into mitosis.

Originally identified as the product of the *cdc2* gene of the fission yeast *Schizosaccharomyces pombe* and the *CDC28* gene in the budding yeast *Saccharomyces cerevisiae*, pp34 is now known to be the catalytic subunit of the mitosis-inducing protein kinase known as maturation promoting factor (MPF) or growth-associated H1 kinase (see ref. 2). The other major subunits of MPF are the cyclins[3-7], proteins that accumulate in each interphase and are degraded at the end of each mitosis (reviewed in ref. 8).

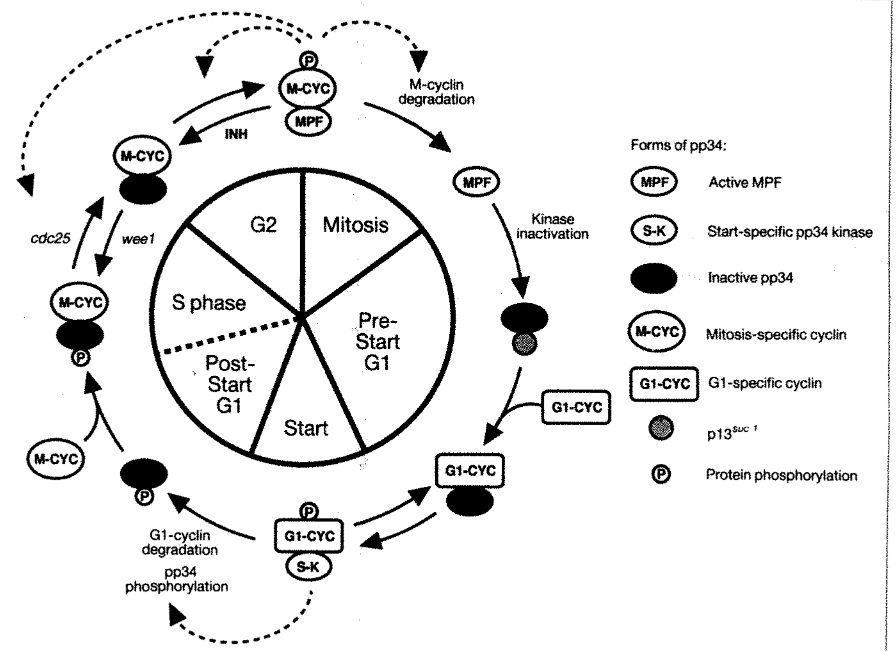
Fission yeast requires *cdc2* activity both for passage through Start and for the induction of mitosis. In this organism, entry into mitosis is controlled by the ratio of the activity of two genes: *cdc25*, which stimulates entry into mitosis, and *wee1*, which inhibits it and has homology to known protein kinases[9]. In late interphase pp34 is phosphorylated on both threonine and tyrosine[10]. The activation of MPF, both *in vivo* and *in vitro*, is accompanied by dephosphorylation of both amino acids[11-14], leading to the idea that the dephosphorylation of pp34 regulates the activation of MPF. Gould and Nurse's new data from fission yeast strongly support this hypothesis.

Their findings can be summarized as follows. In fission yeast whose cell cycle has been arrested in G2 by a temperature-sensitive *cdc25* mutant, pp34 is phosphorylated on tyrosine 15 and on an unidentified threonine. When the cells are returned to the permissive temperature the abundance of both phosphoamino acids declines and the cells enter mitosis. When phenylalanine is substituted for tyrosine 15 of pp34, the mutant protein is no longer a substrate for tyrosine phosphorylation. Strains that carry this mutation grow poorly, even though they divide at a much smaller size than wild-type cells. Moreover, they exhibit a large number of aberrant cell divisions that are presumed to result from premature entry into mitosis. (This phenotype is strongly reminiscent of the 'mitotic catastrophe' induced by the over-expression of *cdc25* in strains carrying mutations in *wee1* (ref. 15).) Finally, the mutant strain can still exit from mitosis, demonstrating that tyrosine phosphorylation of pp34 is not required for this step.

These elegant experiments suggest that the antagonistic effects of *wee1* and *cdc25* may act to control the tyrosine phosphorylation of pp34. This mechanism for controlling entry into mitosis is probably phylogenetically conserved, as homologues of *cdc25* have been found in *Drosophila*[16] and *S. cerevisiae*[17]. Unfortunately, the simplest scheme — that the *wee1* product is the pp34 tyrosine kinase and the *cdc25* product is the pp34 tyrosine phosphatase — is unlikely to be true for several reasons. First, the sequence of the *wee1* product resemble serine/threonine kinases rather than tyrosine kinases. Second, the sequence of the *cdc25* product does not resemble known protein phosphatases. Finally, the dramatic effect of overexpressing *cdc25* in strains mutant for *wee1* (ref. 15) shows that *cdc25* must do more than simply reverse the inhibitory effects of *wee1*. It will clearly be of interest to determine whether *wee1* activity is required for the tyrosine phosphorylation of pp34 and the effect of mutating the sites of threonine phosphorylation on pp34. The ability of fission yeast to divide normally in the absence of both *cdc25* and *wee1* functions[9] indicates that there are additional controls on the activation of MPF that remain to be discovered.

How many biochemical steps are involved in activating the mitosis-specific activity of pp34? Two that we know of are cyclin accumulation[18,19] and pp34 dephosphorylation[1, 11-14]. In addition, in sea urchins an increase in cyclin phosphorylation parallels the activation of MPF[7]. Thus, in the illustrated model, cyclin (M-CYC) binding to pp34 is required to allow the removal of tyrosine and threonine phosphates from pp34, and the subsequent phosphorylation of cyclin that yields active MPF. The level of phosphorylation of pp34 and cyclin is determined by the balance between kinases and phosphatase activities, so many different regulatory signals can act on this part of the cycle. The *cdc25* and *wee1* genes are shown as stimulating, respectively, the dephosphorylation and phosphorylation of pp34.

In the model, the dephosphorylation of cyclin is mediated by INH, an inhibitor of MPF activation that has been postulated to be a phosphatase[20]. MPF catalyses the activation of inactive forms of MPF[1,12,20] and the incorporation of phosphate into the cyclin component of MPF[4,5,7], indicating that it may be the physiological cyclin kinase. The model also shows MPF acting indirectly to increase the rate of tyrosine dephosphorylation. These autocatalytic steps would ensure that the rise in the levels of MPF is rapid and not easily



A model of the cycle of pp34 (outer ring) during the cell cycle. The pp34 molecule lacks kinase activity during most of the cell cycle but becomes active as MPF at mitosis and as a Start-specific kinase at Start as a result of association with phosphorylated cyclins. Note that the active forms can also stimulate their own activation and/or inactivation (----▶). The dashed line between post-Start G1 and S phases indicates that no change in pp34 accompanies the onset of replication.

reversible. It is not known whether pp34 dephosphorylation requires cyclin accumulation or pp34 activity, but this question can be addressed experimentally in both intact fission yeast and frog egg extracts.

How is MPF turned off at the end of mitosis? Cyclin degradation is necessary for the exit from mitosis[21], but the existence of cyclin-free forms of MPF *in vitro*[12], and the transient existence of H1 kinase activity after the destruction of cyclin *in vivo*[22], suggest that cyclin degradation is not sufficient for the inactivation of MPF. One candidate member of the inactivation pathway is p13^{suc1}, the product of the fission yeast *suc1* gene. Not only are cells deleted for this gene unable to inactivate MPF[22], but the gene product binds pp34, stabilizes the kinase activity of certain temperature-sensitive alleles of *cdc2* (refs 5, 22), and can block the dephosphorylation of pp34 in frog extracts[14]. Perhaps, in the absence of cyclin, p13^{suc1} binding to pp34 destroys the catalytically active conformation of the molecule or renders it a substrate for some inactivating activity. In both *Aspergillus* and fission yeast, mutants in type 1 phosphatase genes are defective in the exit from mitosis (see ref. 23). It is not yet known whether the defect in these strains is in the inactivation of MPF or in the removal of phosphate from molecules whose phosphorylation is induced by active MPF.

In mammalian cells and budding yeast, Start is the point in the cell cycle where cells monitor cell size and nutrient availability. What role does pp34 play in this cell-cycle transition? Recent evidence from the fission yeast[24-26] suggests there are G1-specific cyclins (G1-CYC in the model) that activate a Start-specific protein kinase activity of pp34 in much the same way as the mitosis-specific cyclins activate MPF. The mechanisms that regulate Start, the biochemical events that are the manifestation of passage through Start, and the substrates of the Start-specific activity of pp34 are mostly unknown. In exponentially growing HeLa cells, the level of phosphotyrosine in pp34 does not increase until the onset of S phase[11]. This observation raises the possibility that tyrosine phosphorylation of pp34 is a consequence of passage through Start and requires the Start-specific activity of *cdc2*, just as the tyrosine dephosphorylation at mitosis is postulated to require the mitosis-specific activity of *cdc2*. Thus, tyrosine phosphorylation of pp34 would serve as a marker for those cells that had passed through Start, as well as regulating entry into mitosis.

If complete removal of pp34 phosphotyrosine is dependent on high levels of MPF activity this stigma of passage through Start could not be removed without entry into mitosis. Such a scheme provides a biochemical means for the cell to remember where it is in the cell cycle and thus ensure a regular alternation of Start and mitosis. Finally, if the removal of phosphotyrosine from pp34 is dependent on the completion of DNA synthesis, mitosis could not start until DNA synthesis had finished. ☐

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1. Gould, K.L. & Nurse, P. *Nature* **342**, 39–45 (1989).
2. Murray, A.W. *Nature* **335**, 207–208 (1988).
3. Pines, J. & Hunter, T. *Cell* **58**, 833–846 (1989).
4. Draetta, G. *et al. Cell* **56**, 829–838 (1989).
5. Booher, R.N., Alfa, C.E., Hyams, J.S. & Beach, D.H. *Cell* **58**, 485–497 (1989).
6. Giordano, A. *et al. Cell* **58**, 981–990 (1989).
7. Meijer, L. *et al. EMBO J.* **8**, 2275–2282 (1989).
8. Murray, A.W. *Nature* **326**, 542–543 (1987).
9. Russell, P. & Nurse, P. *Cell* **49**, 559–567 (1987).
10. Draetta, G. *et al. Nature* **336**, 738–744 (1988).
11. Morla, A.O., Draetta, G., Beach, D. & Wang, J.Y.J. *Cell* **58**, 193–203 (1989).
12. Labbe, J.C. *et al. Cell* **57**, 253–263 (1989).
13. Gautier, J., Matsukawa, T., Nurse, P. & Maller, J. *Nature* **339**, 626–629 (1989).
14. Dunphy, W.G. & Newport, J.W. *Cell* **58**, 181–191 (1989).
15. Russell, P. & Nurse, P. *Cell* **45**, 145-153 (1986).
16. Edgar, B.A. & O'Farrell, P.H. *Cell* **57**, 177–187 (1989).
17. Russell, P., Moreno, S. & Reed, S.I. *Cell* **57**, 295–303 (1989).
18. Murray, A.W. & Kirschner, M.W. *Nature* **339**, 275–280 (1989).
19. Minshull, J., Blow, J. & Hunt, T. *Cell* **56**, 947–956 (1989).
20. Cyert, M.S. & Kirschner, M.W. *Cell* **53**, 185–195 (1988).
21. Murray, A.W., Solomon, M.J. & Kirschner, M.W. *Nature* **339**, 280–286 (1989).
22. Moreno, S., Hayles, J. & Nurse, P. *Cell* **58**, 361–372 (1989).
23. Cyert, M.S. & Thorner, J. *Cell* **57**, 891–893 (1989).
24. Cross, F. *Molec. cell. Biol.* **8**, 4675–4684 (1988).
25. Nash, R., Tokiwa, G., Anand, S., Erickson, K. & Futcher, A.B. *EMBO J.* **7**, 4335–4346 (1988).
26. Hadwiger, J.A., Wittenberg, C., Richardson, H.E., de Barros-Lopes, M. & Reed, S.I. *Proc. natn. Acad. Sci. U.S.A.* **86**, 6255–6259 (1989).

ATMOSPHERIC CIRCULATION

Variations without forcing

Marvin A. Geller

ATMOSPHERIC conditions can vary over a vast spectrum of timescales — extending up to the Milankovic cycles operating over millenia and forced by variations in the Earth's orbit. But James and James warn on page 53 of this issue[1] against reading too much into correlations between climate cycles and the periods of plausible forcing mechanisms. In particular, they show that one 11-year circulation cycle that appears to be related to the 11-year solar cycle[2] can arise naturally from the internal dynamics of the atmosphere.

James and James base their warning on computer simulations of the climate for timescales of 100 years. The physical inputs of the simulation were deliberately simplified to facilitate the long integration times, but included steady solar heating, the effects of friction on the atmospheric circulation, effects due to the Earth's rotation and, in one case, the seasonal cycle. Other variations in external forcing are deliberately excluded. The important difference from previous simulations is the allowance for nonlinear effects in such long integrations.

The resulting atmospheric circulation was examined for periodic variations. This is best illustrated in the spectra in Figs 2–4, on pages 54–55 of this issue, which show the variation of a particular parameter of the circulation — the (0,1) vorticity coefficient which reflects the excess of atmospheric rotation over the Earth's. Despite the lack of any long-period forcing, the circulation varies strongly for periods longer than a year, the strongest response being at around ten years. This is apparent in the simulations that include annual forcing (Figs 2 and 3) and, most significantly, in the simulation in which this was excluded (Fig. 4). This demonstrates, James and James conclude, that the presence of decadal variations in the atmosphere does not imply that a forcing mechanism of similar period is at work, a conclusion anticipated in long-term simulations of temperature changes from a general circulation model[3].

The new results imply that this type of variability can occur as a result of long-lived, non-random effects in the nonlinear atmospheric flow. Unlike linear systems, nonlinear systems can respond to internal and external forcings over periods much longer than those directly being forced. The results of James and James suggest that, although the internal atmospheric instabilities generate internal variability in the fluid flow on timescales of the order of ten days, these are organized by the nonlinear flow into long-lived structures that show variations over timescales as great as ten years: the decadal variations are internally produced.

This result is very relevant to suggestions of a connection between climate and the solar cycle. Two years ago, Labitzke and van Loon[2] published a provocative paper demonstrating excellent correlation between atmospheric variability at stratospheric levels during the winter months and solar activity. This becomes apparent when the winter data are subdivided into two sets according to the phase of the quasibiennial oscillation (QBO) in the lower-stratospheric tropical wind patterns. The authors have since demonstrated that these strong correlations also exist at tropospheric levels and during other seasons[4,5].

There have been several attempts to check the validity of Labitzke and van Loon's statistical approaches. In most cases, their procedures are considered to have passed these tests. In fact, the statistical associations have been sufficiently convincing that some operational forecasts of seasonal mean climate have begun to use the results in making these forecasts[6]. At the same time, strong scepticism has also been expressed about the idea that the correlations imply that solar activity controls the lower atmosphere. The results of the James and James paper provide a good perspective in which to examine this situation.

Van Loon and Labitzke[4] state the issue very well at the end of their paper:

> Some of our colleagues have suggested that what we have detected is a strong tendency for large-amplitude atmospheric variability on a decadal timescale which shows when the observations are filtered through the Quasi-Biennial Oscillation. This is of course a possibility, but such variability has no obvious explanation either. We find it remarkable if an internal oscillation in the atmosphere on a decadal time scale happened to be in phase with the 11-year solar cycle during the very 36 years when we describe it.

James and James clearly do suggest an explanation for atmospheric variability on a decadal timescale: it occurs as a consequence of internal variability. Although it may be improbable that the phase of this internal mode of variability coincides with the phase of solar variability, it is not wildly so. Given that the periods of both variations are about ten years, the probability that they appear to be in phase is about one in five. Furthermore, as both variations tend to be coherent over several cycles, once they are in phase with one another they will tend to stay in phase for several cycles.

The conclusion that Labitzke and van Loon's work implies a large atmospheric response to solar activity forcing has been criticized[7,8] from two additional perspectives. Baldwin and Dunkerton[7] have subjected atmospheric data to the same statistical procedure except they correlate them with simulated harmonics of arbitrary period instead of the solar cycle. They find that correlations equal to or even exceeding those found by Labitzke and van Loon between atmospheric variations and solar activity occur in approximately 5 per cent of these cases. Again, the point is that, viewed over a short number of periods, there is a small but reasonable probability of finding phase matching between two unconnected cycles. This is the flaw in concluding that Labitzke and van Loon's correlations are forced by solar activity.

Barnett[8] shows that there is a significant quasi-biennial variation in tropical sea-surface temperatures, the amplitude of which is modulated over a ten-year period, apparently in phase with the solar cycle. One possibility is that there is a causal relationship between solar variability and the ocean. Or perhaps the decadal atmospheric variability indicated by James and James's results forces the ocean variations. Or the atmosphere and ocean together may display modes of variability that are not present in either individually[9]. Thus, the atmosphere–ocean system has many ways of producing decadal variations internally.

No physical mechanism capable of quantitatively explaining any effect of solar activity on the lower atmosphere has been identified, despite many years of research: this is because solar activity can modulate only weakly the energy put into the atmosphere. Without a plausible mechanism, it is much more likely that we are seeing physically unrelated, though correlated, internal variations in the Sun and internal variations in the atmosphere or ocean–atmosphere system. ☐

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1. James, I. N. & James, P. M. *Nature* **341**, 53–55 (1989).
2. Labitzke, K. & van Loon, H. *J. atmos. terr. Phys.* **50**, 197–206 (1988).
3. Hansen, J. & Lebedeff, S. *J. geophys. Res.* **92**, 13345–13372 (1987).
4. van Loon, H. & Labitzke, K. *J. Climate* **1**, 905–920 (1988).
5. Labitzke, K. & van Loon, H. *J. Climate* **2**, 554–565 (1989).
6. Kerr, R. A. *Science* **242**, 1124–1125 (1988).
7. Baldwin, M. P. & Dunkerton, T. J. *Geophys. Res. Lett.* **16**, 863–866 (1989).
8. Barnett, T. P. *Geophys. Res. Lett.* **16**, 803–806 (1989).
9. Schopf, P. S. & Suarez, M. J. *J. atmos. Sci.* **45**, 549–566 (1988).

CALCIUM SIGNALLING

Receptor kinships revealed

Donald L. Gill

JUST six years have passed since the action of inositol 1,4,5-trisphosphate (InsP$_3$) on the release of intracellular Ca^{2+} was first reported[1]. Since then InsP$_3$ has become recognized as a universal 'second messenger' that is released into the cell cytosol following the breakdown of phosphatidylinositol 4,5-bisphosphate in response to cell surface receptor-mediated activation of phospholipase C. InsP$_3$ is then either further metabolized (see ref. 2) or it interacts with the receptors that mediate intracellular Ca^{2+} release, but whose nature has remained obscure. Recently, Supattapone *et al.*[3] isolated an InsP$_3$-binding protein from the cerebellum that had properties consistent with the receptor. Now an ensemble of independent reports from the laboratories of Mikoshiba[4], Snyder[5] (on pages 32 and 87 of this issue) and Südhof[6], provide important sequence and structural information on this protein and compelling evidence that the same molecule mediates both InsP$_3$-binding and the release of Ca^{2+} from the endoplasmic reticulum. Surprisingly, the structure of the InsP$_3$ receptor is also shown to be strikingly similar to the recently cloned ryanodine receptor (RR)[7], the Ca^{2+} channel of the sarcoplasmic reticulum of skeletal muscle.

An interesting history precedes identification of the InsP$_3$ receptor; the same protein has been described independently several times from as early as 1976 (see refs 4 and 5). The extraordinary abundance of the protein in cerebellar Purkinje cells provides an explanation for this unusual degree of attention. It is a membrane glycoprotein with a relative molecular mass (M_r) of 250,000 (250K) which binds to both heparin and concanavalin A (enabling an effective purification scheme[3]), and is a major substrate for cyclic AMP-dependent phosphorylation[8]. Various names have been assigned to the protein; Mikoshiba and colleagues refer to it as P$_{400}$ (based on a somewhat spurious original size determination), whereas it is also referred to as PCPP-260 (ref. 8) and GP-A (ref. 9). Henceforth, it seems we may now have confidence in referring to it as the functional InsP$_3$receptor (InsP$_3$R).

Furuichi *et al.*[4] in Mikoshiba's laboratory used three monoclonal antibodies to the purified P$_{400}$ protein to identify complementary DNAs within mouse cerebellum cDNA libraries that expressed the protein. Eventually, the entire cDNA sequence was obtained, revealing an open reading frame encoding a protein of 2,749 amino acids of theoretical M_r 313K. From hydropathy profiles, the authors conclude that the protein contains up to nine membrane-spanning domains, but from immunolocalization studies not presented a very large N-terminal region of the protein is on the cytoplasmic face of the endoplasmic reticulum (ER), so that it is only a cluster of up to seven transmembrane domains close to the C terminus that is likely to exist. Localization of the C terminus on the luminal side of the ER was made possible by a nearby epitope and by evidence of glycosylation in the same region. The large cytoplasmic domain probably contains the InsP$_3$ recognition site (and regulatory elements) with the transmembrane domains providing the Ca^{2+}-translocating region; however, assignment of functional domains is still tentative.

Quite independently, Mignery *et al.*[6] in Südhof's laboratory made several rather intuitive deductions. They noticed that the recently reported[10] partial sequence of a major Purkinje cell transcript named PCD6 (containing an open reading frame encoding 500 amino acids) was for a protein containing several transmembrane domains and perhaps, therefore, an abundant channel protein. With antibodies to the C-terminal peptide, Mignery

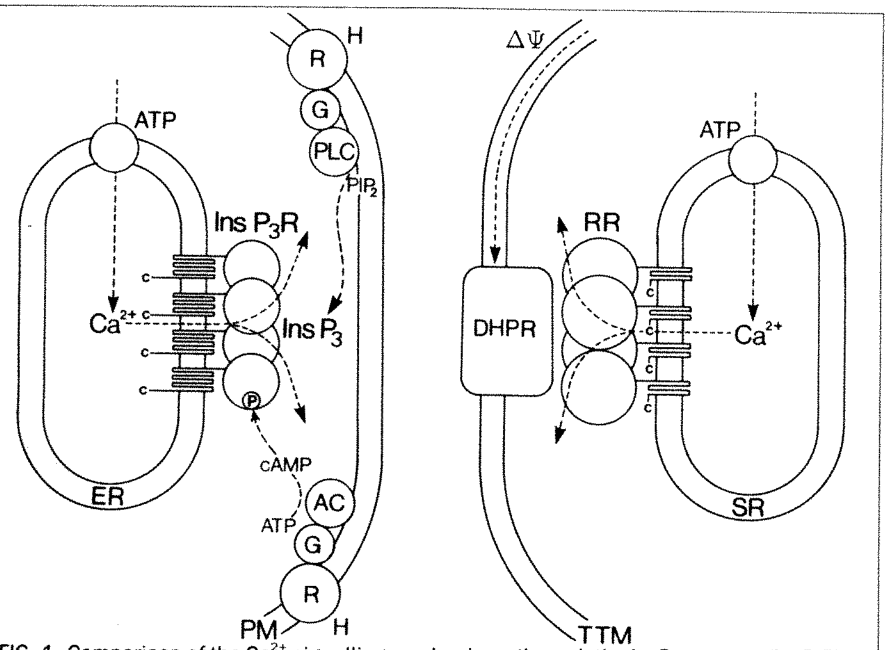

FIG. 1 Comparison of the Ca^{2+} signalling mechanisms through the $InsP_3$ receptor ($InsP_3R$) in nonmuscle tissue (left) and the ryanodine receptor (RR) in skeletal muscle (right). In nonmuscle, hormones (H) binding to plasma membrane (PM) receptors (R) coupled to G proteins (G) activate phospholipase C (PLC) and the breakdown of phosphatidylinositol 4,5-bisphosphate (PIP_2) to release inositol 1,4,5-trisphosphate ($InsP_3$) into the cytosol. The action of $InsP_3$ on Ca^{2+} release from endoplasmic reticulum (ER) is mediated by the $InsP_3R$, depicted here as being tetrameric (although of a size suggesting four molecules, the number of subunits in the $InsP_3R$ complex has not been determined). In addition, cAMP, produced by hormonal modification of adenylyl cyclase (AC), activates phosphorylation of the $InsP_3R$ and inhibits $InsP_3$-induced activation of Ca^{2+} release[19]. In skeletal muscle, the initial signal is an electrical depolarization ($\Delta\Psi$) of the T-tubule membrane (TTM), which is detected by the voltage-dependent dihydropyridine-sensitive receptor (DHPR). The signal is believed to be modulated by the RR N terminus (in close contact with the DHPR) into opening of a channel within the core of the RR tetrameric structure[7,11]. In both systems, the end result is Ca^{2+} release into the cytosol. Note that the C terminus (C) of the $InsP_3$ the is luminal, whereas that of the RR is believed to be cytoplasmic[7].

et al. rapidly identified the sequence as part of a heparin-binding protein, of M_r 260K, whose identity with the protein described by Supattapone *et al.*[3] was confirmed by its ability to bind $InsP_3$. Indeed, its 500-amino-acid sequence differs by only one amino acid from the C-terminal sequence obtained by Furuichi *et al.*[4].

Is this molecule the functional receptor or merely an $InsP_3$-binding constituent of a larger Ca^{2+}-releasing complex containing additional proteins? The answer is convincingly provided by Ferris *et al.*[5] in the Snyder laboratory, who have now demonstrated that the purified molecule, incorporated into liposomes, mediates Ca^{2+} release in response to $InsP_3$ concentrations that saturate the binding site. Perhaps most satisfying is the pharmacological specificity: Ins(1,3,4)P_3, a metabolite of Ins(1,4,5)P_3 with very little activity *in vivo*, is completely ineffective in inducing Ca^{2+} release in the reconstituted system. Although additional proteins are apparently not required for $InsP_3$-induced transport, evidence discussed below suggests that the functional receptor is a multimer of the protein now described.

It comes as a major revelation that the $InsP_3R$ and the RR have similar structures. Over a region of 134 amino acids (residues 2,541–2,674) in the C-terminal region of the $InsP_3R$ there is 46 per cent identity (64 per cent including conservative changes) with the C-terminal region of the RR. In both cases the region contains the final transmembrane domain. The adjacent transmembrane domains and smaller regions scattered throughout the large N-terminal region of the two molecules are also similar. The total number of possible transmembrane domains in the $InsP_3$ receptor is in some dispute. As opposed to the seven suggested by Furuichi *et al.*[4] (which account for oppositely orientated N and C termini), Mignery *et al.*[6] identify four (the same as the RR), but in either case they all occur within the last 475 C-terminal amino acids. Regardless of the number of transmembrane domains, the overall topology of the $InsP_3R$, including a large cytoplasmic N-terminal domain and transmembrane domains clustered close to the C terminus, closely resembles the RR, as shown in Fig. 1. However, the evidence suggests that the $InsP_3R$ C terminus is luminal (see above), whereas the RR C terminus is apparently cytoplasmic. Considering the structural similarity between the C termini and two adjacent membrane-spanning domains, it seems strange that the two regions should be oriented oppositely across the membrane, as it is likely that such regions have important functional correlates (for example, ion translocation).

Another important similarity between the $InsP_3R$ and the RR is that both associate into multimeric structures. There is agreement[3,4,6] that the M_r of solubilized $InsP_3R$ is around 1,000K, suggesting a tetrameric structure, as shown in Fig. 1. This is particularly reminiscent of the RR, which is believed to function in sarcoplasmic reticulum junctions as a tetramer in an approximately square configuration[11], the four subunits possibly surrounding a central Ca^{2+} channel. Indeed, Furuichi *et al.* refer to electron-microscopic evidence for the existence of just such a square-shaped multimeric structure for the $InsP_3R$. Moreover, a multimeric arrangement is indicated by certain functional studies. Thus, there is good evidence that $InsP_3$ receptors function cooperatively, occupancy of three or more sites contributing to channel opening[12]; also, the high antagonistic potency of oligomeric heparin (more than 5 nm in length) on $InsP_3$ effects could reflect a close proximity between groups of $InsP_3R$ molecules[13]. Further assessment of tertiary and quaternary structure of the $InsP_3R$ may reveal much more about the function and control of Ca^{2+} release.

What is the function of the very large N-terminal domain of the $InsP_3R$? By analogy (and limited sequence homology) to the N-terminal 'foot' of the RR, which is believed to interact with the dihydropyridine-sensitive receptor in fast muscle (Fig. 1), could this domain be involved in connections to another controlling

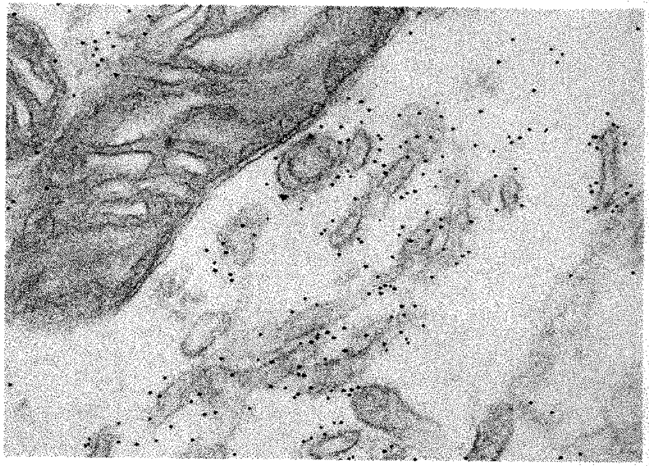

FIG. 2 Localization of the putative $InsP_3$ receptor in a cerebellar Purkinje cell. Electron micrograph of a cell treated with a gold-labelled antibody to a peptide of the receptor, shows that gold particles are confined to the endoplasmic reticulum. (From ref. 6.)

protein? The lack of any strict organizational configuration of InsP$_3$-responsive organelles, compared with the precise organization of sarcosplasmic reticulum feet, would militate against such a role. This raises the question of exactly where within cells InsP$_3$ receptors are localized. On this point the results are not so consistent. Recent immunocytochemical studies[14], show that the ER is a principal site of InsP$_3$R, particularly rough ER (and nuclear envelope), but also peripheral smooth ER (sometimes at locations close to the plasma membrane). The immunolocalization of Mignery *et al.*[6] is generally consistent with this, although showing more uniform ER labelling (Fig. 2). But Furuichi *et al.*[4] provide evidence for additional localization in the plasma membrane, a result that supports the functional data of Kuno and Gardner[15] for InsP$_3$R-mediated Ca^{2+} translocation across this membrane.

The interesting heterogeneity of InsP$_3$R locations does not support the idea of a single specialized InsP$_3$-responsive organelle (the calciosome) within cells as suggested by Volpe *et al.*[16]. We must also consider the occurrence of complex and dynamic interactions between InsP$_3$-sensitive and -insensitive organelles mediated by intracellular G proteins[17]; such changes may considerably alter the size and location of InsP$_3$-responsive Ca^{2+} pools, and hence the vicinity of InsP$_3$ receptors.

Lastly, we should consider that much of what has been discussed relates to Purkinje cells, where the massive InsP$_3$R density is apparently designed to facilitate extensive granule cell interactions, which are mediated by glutamate receptors coupled to phosphoinositide breakdown[18] (the cerebellum is thus almost a 'skeletal non-muscle' tissue in terms of specialized Ca^{2+} signalling). Whereas indentical InsP$_3$ receptors appear to exist in most other cell types (S.H. Snyder, personal communication), their organization, control, and distribution may be quite different. □

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1. Streb, H., Irvine, R.F., Berridge, M.J. & Schulz, I. *Nature* **306**, 67–69 (1983).
2. Berridge, M.J. & Irvine, R.F. *Nature* **341**, 197–205 (1989).
3. Supattapone, S., Worley, P.F., Baraban, J.M. & Snyder, S.H. *J. biol. Chem.* **263**, 1530–1534 (1988).
4. Furuichi, T., Yoshikawa, S., Miyawaki, A., Wada, K., Maeda., N. & Mikoshiba, K. *Nature* **342**, 32–38 (1989).
5. Ferris, C.D., Huganir, R.L. Supattapone, S. & Snyder, S.H. *Nature* **342**, 87–89 (1989).
6. Mignery, G.A., Südhof, T.C., Takei., K. & De Camilli, P. *Nature* (in the press).
7. Takeshima, H. *et al. Nature* **339**, 439–445 (1989).
8. Walaas, S.I., Nairn, A.C. & Greengard, P.J. *Neurosci.* **6**, 954–961 (1986).
9. Groswald, D.E. & Kelly, P.T. *J. Neurochem.* **42**, 534–546 (1984).
10. Nordquist, D.T., Kozak, C.A. & Orr, H.T. *J. Neurosci.* **8**, 4780–4789 (1989).
11. Lai, F.A., Erickson, H.P., Rousseau, E., Liu, Q-Y. & Meissner, G. *Nature* **331**, 315–319 (1988).
12. Meyer, T., Holowka, D. & Stryer, L. *Science* **240**, 653–656 (1988).
13. Ghosh, T.K., Eis, P.S., Mullaney, J.M., Ebert, C.L. & Gill, D.L. *J. biol. Chem.* **263**, 11075–11079 (1988).
14. Ross, C.A. *et al. Nature* **339**, 468–470 (1989).
15. Kuno, M. & Gardner, P. *Nature* **326**, 301–304 (1987).
16. Volpe, P. *et al. Proc. natn. Acad. Sci. U.S.A.* **85**, 1091–1095 (1988).
17. Ghosh, T.K., Mullaney, J.M., Tarazi, F.I. & Gill, D.L. *Nature* **340**, 236–239 (1989).
18. Blackstone, C.D., Supattapone, S. & Snyder, S.H. *Proc. natn. Acad. Sci. U.S.A.* **86**, 4316–4320 (1989).
19. Supattapone, S. *et al. Proc. natn. Acad. Sci. U.S.A.* **85**, 8747–8750 (1988).

OCEAN DRILLING PROGRAM

Arc volcanism and rifting

*Leg 126 shipboard scientific party**

ACTIVE island-arc volcanoes, noted for their explosive eruptions, characterize the margins of converging oceanic plates. Our knowledge of the nature of volcanism and sedimentation in these areas, and how 'forearc' and 'backarc' basins are formed, has been significantly advanced by recent ocean drilling in the central Izu–Bonin Arc, south of Japan (Fig. 1). Our initial results show that arc rifting is geologically rapid, requiring only two to three million years, and can occur on either or both sides of the island arc. The rifting stage is accompanied by intense volcanism and rapid sedimentation, whereas during subsequent backarc spreading the arc may be relatively quiescent. Volcanism, even explosive eruption, is not restricted to the island arc, but may occur in deep water to either side.

The Izu–Bonin volcanic arc is a product of 50 million years (Myr) of subduction of Pacific lithosphere. Backarc spreading between 25 and 15 Myr ago in the Shikoku Basin separated the formerly contiguous Kyushu–Palau Ridge from the still active arc (Fig. 1). We drilled in the Sumisu Rift to investigate the geological processes associated with renewed rifting of the arc (before backarc spreading). We also drilled in the forearc basin and discovered that it, too, was formed by rifting, in the mid-Oligocene (28–31 Myr ago).

Four cycles of explosive volcanic activity in the last 250 thousand years deposited glassy and pumiceous silts, sands and gravels which we recovered at the Sumisu Rift sites 790 and 791. This material probably erupted from the nearby Sumisu and/or South Sumisu calderas. The pumiceous unit overlies nannofossil-rich clays, with thin ash beds and scattered pumice and scoria clasts, that date back to 1.1 Myr ago. Sedimentation rates increase exponentially from 80 and 300 m Myr^{-1} at 1 Myr ago to 1,000 and 2,250 m Myr^{-1} in the past 100 thousand years at sites 790 and 791, respectively. Benthic foraminifers are consistent with deposition in the present 2-km water depths during the past 730 thousand years and at somewhat deeper levels previously. These data require rapid, differential and accelerating subsidence and sedimentation in the rift half graben. In contrast, only about 30 m of pumiceous gravel have been deposited in the past 275 thousand years, above a major unconformity at the rift margin site 788. The unconformity is the result of margin uplift of between 200–1,700 m (according to benthic foraminiferal data) since the deposition of the underlying pumiceous units 2.35–3.56 Myr ago. The rift formed very quickly, beginning 2 ± 0.5 Myr ago.

a

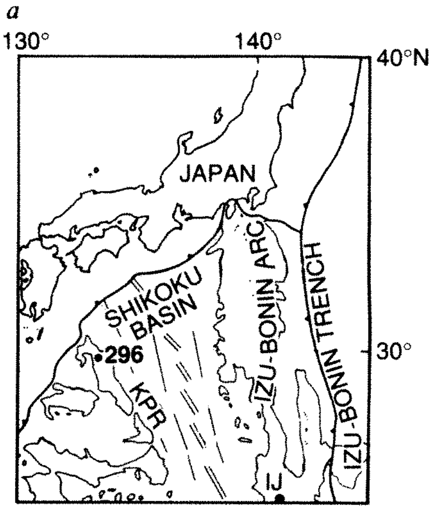
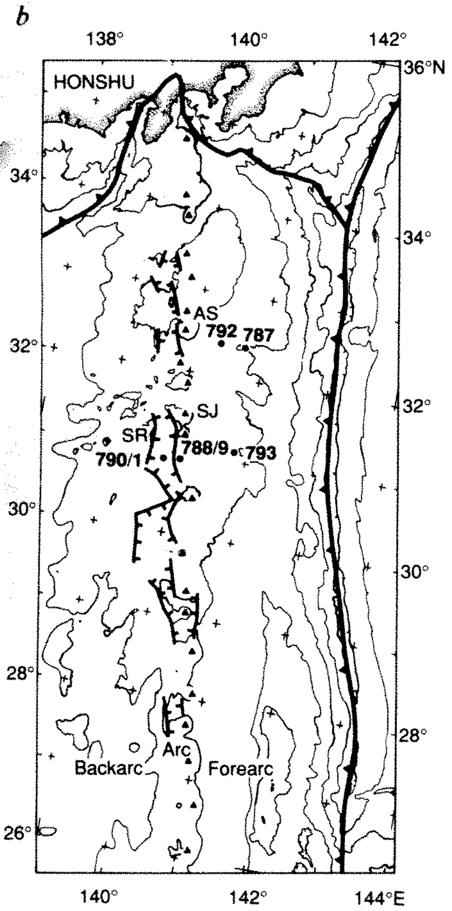


FIG. 1 *a*, Active plate boundaries and relict spreading centres in the Philippine Sea region. Barbed lines indicate subduction zones and double lines locate relict spreading centres. Basins and ridges are outlined by the 4-km bathymetric contour, except for the Izu–Bonin arc, which is outlined by the 3-km contour. Magnetic anomalies 6 and 6B are shown by single thin lines in Shikoku Basin. IJ=Iwo Jima; KPR=Kyushu–Palau Ridge; 296=DSDP site 296. *b*, Bathymetric map showing location of Leg 126 sites. Thick barbed lines locate subduction zones and lines with tic marks indicate rifts. SR=Sumisu Rift; AS=Aoga Shima; SJ=Sumisu Jima. ▶

A 135-m-thick basalt unit at site 791 probably resulted from submarine fountaining of hydrous lava deeper than 1,500 m below sea level. This unit is composed of clastic basalt froth: both the glassy matrix and clasts are honeycombed with small vesicles, totalling 30–40 per cent porosity. All the tholeiitic basalts at sites 790 and 791 are highly vesicular (20–30 per cent), despite their eruption in deep water, owing to their high pre-eruption water contents. The rift basement basalts may be distinguished from Izu–Bonin arc basalts by their lower barium contents (below 30 parts per million) and higher TiO$_2$ contents (above 0.95 per cent) relative to MgO, zirconium and vanadium.



The basalts at site 791 have concentrations of barium and rubidium as low as those in mid-ocean ridge basalts and in the most mature backarc basins, indicating that basalts' compositions can lose their arc-like geochemical characteristics very early in the rifting stage of backarc basin development. The lowermost rocks at site 791 are arc-derived pyroclastics. Their pumice shards have a eutaxitic texture suggesting hot emplacement, but it is uncertain whether they were emplaced before or during rifting.

Intra-oceanic forearc basins have been avoided by previous drilling legs because of their large sediment thickness. Their stratigraphy, however, records the history of forearc sedimentation, arc volcanism, microstructural deformation and plate motion. To recover this record, and to sample the underlying basement for the first time, we drilled at three sites in the Izu–Bonin forearc (Fig. 2). Studying the basement at the centre of the basin involved drilling the Ocean Drilling Program's deepest hole yet (793B), to 1,682 m below the sea floor.

The three forearc sites documented a characteristic pattern of volcanogenic input: extremely rapid activity before 28 Myr ago; moderate between 28 and 24 Myr ago; minimal between 24 and 13 Myr ago; and moderate and increasingly fast from 13 Myr ago to the present. This pattern is similar to that observed previously at site 296 on the northern Kyushu–Palau remnant arc ridge (Fig. 1). The early and early-middle Miocene minimum in volcanism corresponds in time with the backarc opening of the Shikoku Basin. This interval is represented in the forearc by nannofossil-rich claystones and nannofossil chalks, quite unlike the surrounding turbidite sequences.

The 200 ash layers (or more) in the upper unit at site 792 are evidence of continuing explosive volcanism since 3 Myr ago. Together with the volcanic record from sites 788–791, they indicate that the Pliocene-to-recent pre- and synrift volcanism and sedimentation along the volcanic front is dominated by rhyolite pumice eruptions. There is no evidence during this time of igneous vents or lava flows between the central Izu–Bonin arc volcanoes, in contrast to arcs such as Japan and the Cascades.

Given the known presence of middle Eocene volcanic highs on both sides of the forearc basin, a big surprise was the lack of Eocene or mid- to lowest-Oligocene (45–34 Myr ago) sediments in the basin. At 715 m below sea floor at site 792, we penetrated an angular unconformity that underlies nearly all the north–central forearc basin sediments. The sediments below this unconformity and down to basement are biostratigraphically and magnetostratigraphically dated as younger than 34 Myr old. At site 793, in the centre of the south–central forearc basin, the sediments overlying the igneous basement are 31 Myr old. Clearly there was no basin before this time. We infer that the surrounding Eocene outer arc and frontal arc highs were formerly contiguous, and that the 40–70-km-wide forearc basin formed when they were separated by rifting in the mid-Oligocene. The stretching event probably affected the whole Eocene basement terrain, from the outer-arc high to the now-remnant arc ridge (site 296). The newly formed basin, 4–5 km deep according to benthic foraminiferal data, was rapidly (250–300 m Myr^{-1}) filled with turbidite and debris flow deposits, produced by both concurrent volcanism and erosion of the surrounding highs.

The basement at site 793 includes pillowed and massive lavas and more abundant hyaloclastite breccias. Most of the volcanics belong to a high-Mg series of basaltic andesites with boninitic affinities. Others belong to a low-Mg series with tholeiitic affinities. The basement high penetrated at site 792 may form the edge of one of the Eocene–Oligocene frontal arc highs which, like the volcanoes at the present volcanic front, are separated by 20–80 km. The basement lavas at site

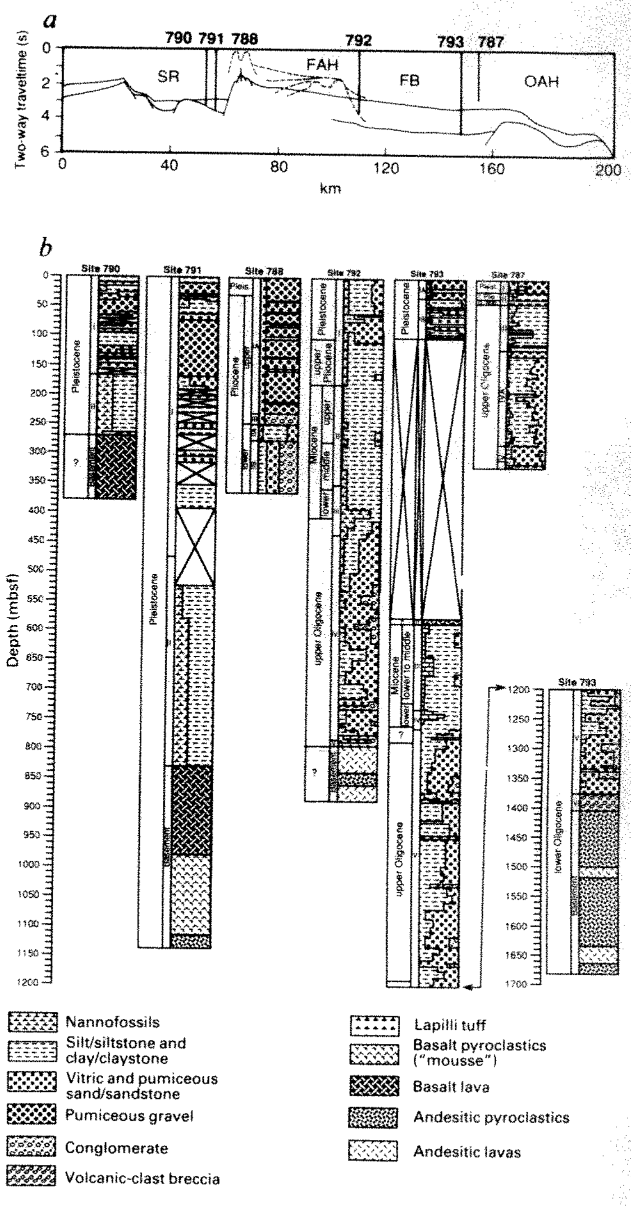


FIG. 2 *a*, Stylized cross-section across the Izu–Bonin arc–forearc showing location of Leg 126 sites. SR=Sumisu Rift; FAH=forearc high; FB=forearc basin; OAH=outer-arc high. *b*, Summary lithostratigraphic columns for Leg 126 sites.

792 are massive flows intercalated with hyaloclastite and breccia layers. They are dominantly porphyritic calcalkaline andesites, but are less enriched in low-field-strength elements such as potassium, rubidium, strontium and barium, than most such arc lavas. A middle Miocene or younger basaltic intrusion, serendipitously cored immediately below the casing at site 793, corroborates pre-cruise multichannel seismic evidence for the presence of sills resulting from Neogene forearc volcanism. Izu–Bonin magmas retained a depleted mantle signature throughout the Tertiary.

The pore-water chemistry was closely monitored to detect hydrothermal circulation, especially in the Sumisu Rift. Although indications of circulating sea water were observed in the forearc basin sites 787 and 792, no evidence of hot water circulation was observed at any site. At sites 792 and 793, however, the most extensively altered pore waters yet recovered by the Drilling Programs were extracted from the volcanogenic sediments below 500 and 800 m below sea floor, respectively. The calcium concentrations are up to 170 and 300 millimolar, respectively. The magnesium concentrations are below detection, sulphate profiles are controlled by the precipitation of gypsum, and at site 793, chloride concentrations exceed 700 millimolar. These highly altered interstitial waters are attributed to the low-temperature alteration of plagioclase and volcanic glass into smectites, zeolites and gypsum, in a virtually closed system, as indicated by the high formation factors. □

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GLOBAL SEA LEVELS

Floodwaters mark sudden rise

M.J. Tooley

GIVEN the wide public concern about the scale of rise in sea level that will occur as a result of the current global warming, many will be intrigued by new studies[1] by J. Shaw of flooding events at the end of the last glacial. From the evidence of erosion and deposition in Saskatchewan, Canada, Shaw concludes that a rise of several metres could have occurred in a few years. Indeed, a catastrophic release of turbulent meltwater from Livingstone Lake, amounting to as much as 8.4×10^4 km^3, could have raised the sea level by 23 cm in as little as a few weeks.

In an attempt to predict the effect of global warming on the sea, earth scientists have analysed the sedimentary record left by past changes. It is clear that the amplitudes of changes vary wildly between different geological eras, although none is as dramatic as that inferred by Shaw.

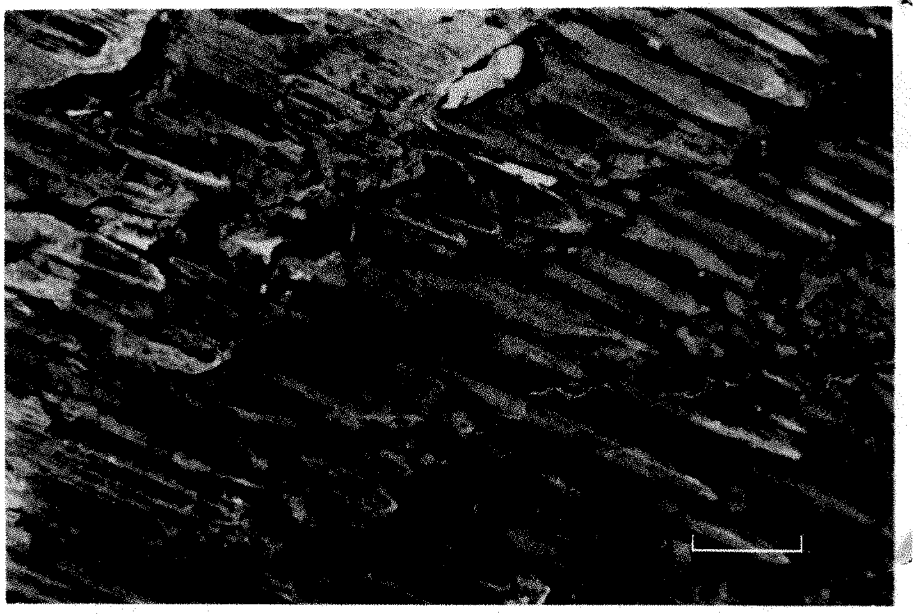
The maximum rate of rise[2] of sea level during the recent geological past (the Holocene or last 10,000 years) did not exceed 22 mm a year. However, this mean value obscures the fact that periods of rapid sea-level change are indicated by analyses of empirical data. In my studies[3,4] in the Ribble estuary, in north-west England, the measured age and altitude of sea-level index points yielded rates of sea-level rise of 34 mm a year about 7,800 years ago; in Morecambe Bay, slightly further north, the rise was 44 mm a year. Corroboration of these rates comes from Limfjord, Denmark, where Petersen[5] showed the rate of sea-level rise to be 46 mm a year between 8,260 and 7,680 years ago. Finally, for Eastern China, Yang *et al.*[6] calculate that the rates for the past 20,000 years have peaked three times at 45, 75 and 20 mm a year, between 15,000 and 11,000 years ago.

Similar rates are forthcoming for the last interglacial age in Europe. At a recent meeting*, H. Streif (Niedersächsisches Landesant Für Bodenforschung, Hannover) concluded that during Eemian II (early in the last interglacial), the sea-level rise was very rapid, possibly 40 mm a year.

These rates of change are several orders of magnitude greater than those calculated for pre-Quaternary periods. For example, during two periods of rapid fall and rise of sea level in the late Cretaceous (over 65 million years ago), rates seem not to have exceeded 9 mm per century[7].

A severe difficulty in these studies is the time resolution for periods of rapid sea-level change, determined with radiometric and luminescent dating techniques. For my Holocene data from coastal Lancashire, the period of rapid sea-level rise is fixed at one site where radiocarbon assays on organic material immediately subjacent to marine silts yielded ages of 7,820±60 and 7,605±85 years. Taking the dates with one standard deviation, there is a 66 per cent chance that the rise of sea level occurred during a period of 360–70 radiocarbon years; with 2 standard deviations, there is a 99 per cent chance that this event occurred within 505 years or instantaneously. Also, the altitudes obtained (−9.6 m and −2.3 m) are present-day measurements and have been affected by sediment consolidation, so that the 7.3 m measured sea-level rise is minimal.

Rapid rates of change are not confined to the oceanic part of the Earth's hydrosphere, and those associated with climate require examination. Bryson *et al.*[8] described the catastrophic disintegration of the Laurentide ice sheet in North America within a couple of centuries about 8,000 years ago, which accounts for the rapid rates of sea-level rise recorded in north-west England and Denmark. The dis-



Aerial photograph of erosional drumlins cut from bedrock in the Northwest Territories, Canada. Shaw uses an analysis of these to infer a massive rate of flooding from the Laurentide icesheet. (Scale bar, 1 km; from ref. 1.)

charge of flood waters, calculated to be 2.0×10^3 km^3, from glacial Lake Missoula is an often-quoted and cyclical event[9]. And Dansgaard *et al.* recently described in *Nature*[10] the abrupt end of the Younger Dryas cold period 10,700 years ago, and concluded that a warming of 7 °C in southern Greenland was completed in 50 years and the climate of the North Atlantic region became milder and less stormy in less than 20 years as the sea-ice cover retreated.

Emiliani *et al.*[11] showed that the discharge of freshwater into the Gulf of Mexico from the Laurentide ice sheet left a clear signature in the ^{18}O isotope record from the sediment cores. Two peak values in the ^{18}O levels about 15,600 and 13,000 years ago now lead Shaw[1] to suggest that they result from two catastrophic meltwater floods from subglacial landform fields. The evidence for such floods comes from an analysis of extensive fields of drumlins — erosional and depositional features — in northern Canada (see figure).

Shaw concludes that rapid changes of sea level may not be a direct consequence of melting arising from climatic causes, but may arise from glacial hydrological causes alone: that is the accumulation and flow of meltwater in reservoirs beneath the ice. Climatic fluctuations then become, in part, a consequence rather than a cause of meltwater floods which could act as a coldwater lid over the North Atlantic ocean, profoundly affecting climatic parameters, such as temperature and evaporation, the stability of the water column and the oxygen isotope signatures in the sedimentary record.

There are implications for the modelling of future sea-level rise, linked, as it is in present investigations, to predicted global temperature rise. The conservative estimates of future sea-level rise given by Hoffman[12] for AD 2025 and AD 2050 are 13.0 and 23.8 cm, respectively — equivalent to a sea-level rise of about 3 mm a year. At a second recent meeting[†] T. Wigley (Univ. East Anglia) concluded that the best estimate of the sea-level rise by AD 2050 was 24–38 cm, or between 3 and 6 mm a year, which is 3–4 times greater than the so-called global rates during the past 100 years calculated from tide gauge data[13]. The impacts of such rates on natural and embanked coasts of the world cannot be underestimated. These rates approach or exceed the critical rate of 5 mm a year calculated from empirical data[14] from the Lincolnshire Fenland when periods of coastline retreat and marine transgression occurred during the past 6,500 years.

None of these rates, however, approaches the rapid rates of sea-level rise recorded from the stratigraphic record of the last interglacial, the culminating stages of the last glaciation and the early part of this interglacial. The present lack of a precise dating technique leads to the conclusion that even the measured rapid rates of sea-level rise between 22 and 75 mm a year may be conservative. The evidence presented by Shaw tends to confirm this conclusion.

Three questions remain, however. First, Boulton[15] concludes that in the absence of evidence for large-scale drumlin formation associated with the catastrophic release of meltwater from beneath glaciers, drumlins are a product of the interaction between a glacier and sediments on its bed. Shaw's explanation of drumlin formation is one of many. Second, Shaw's hypothesis for subglacial, drumlin-forming floods needs to be extended to Fenno–Scandinavia and tested. Third, is there a potential on the Earth now for catastrophic, subglacial floods raising sea level by 23 cm in a few weeks or several metres in a few years? □

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1. Shaw, J. *Geology* **17**, 853–856 (1989).
2. Kwadijk, J. & de Boois, H. (eds) *European Workshop on Inter-related bioclimatic and land use changes: Final report* (National Inst. Public Health and Environmental Protection, Bilthoven, 1989).
3. Tooley, M.J. *Geogrl J.* **140**, 18–42 (1974).
4. Tooley, M.J. *Sea-level Changes* (Clarendon, Oxford, 1978).
5. Petersen, K.S. *Spec. Publs int. Ass. Sediment* **5**, 497–503 (1981).
6. Yang, H., Chen, X. & Xie, Z. in *Late Quaternary Sea-level Changes* (eds Qin, Y. & Zhao, S.) 199–212 (China Ocean, Beijing, 1987).
7. Hancock, J.M. & Kauffman, E.G. *J. geol. Soc. Lond.* **136**, 175–186 (1979).
8. Bryson, R.A., Wendland, K.M., Ives, J.D. & Andrews, J.T. *Arctic Alpine Res.* **1**, 1–14 (1969).
9. Baker, V.R. in *The Channeled Scabland. A guide to the geomorphology of the Columbia Basin, Washington* (eds Baker, V.R. & Nummedal, D.) 59–79 (NASA, 1978).
10. Dansgaard, W., White, J.W.C. & Johnsen, S.J. *Nature* **339**, 532–534 (1989).
11. Emiliani, C., Rooth, C. & Stipp, J.J. *Earth planet. Sci. Lett.* **41**, 159–162 (1978).
12. Hoffman, J.S. in *Greenhouse effect and sea-level rise: a challenge for this generation* (eds Barth, M.C. & Titus, J.G.) 79–103 (van Nostrand Reinhold, New York, 1984).
13. Gornitz, V. & Lebedeff, S. in *Sea-level changes and coastal evolution, Spec. Publ.* no. 41 (Soc. Palaeont. Miner., Tulsa, Oklahoma, 1987).
14. Shennan, I. in *The Wash and its environment* (eds Doody, P. & Barnett, B.) 77–90 (Nature Conservancy Council, Peterborough, 1987).
15. Boulton, G.S. in *Drumlin Symposium* (eds Menzies, J. & Rose, J.) 25–80 (A.A. Balkema, Rotterdam, 1987).

[*] Meeting of INQUA Northwestern Europe Shorelines Subcommission, Tallinn, Estonia, 18–22 September 1989.

[†] European Programme on Climatic Hazards annual meeting, Cork, Ireland, 3 October 1989.

Erratum

It should have been acknowledged that the figure accompanying the News and Views article in *Nature* **341**, 22–23; 1989 by James Scott was based on an unpublished figure of K. A. Hajjar and R. L. Nachman. The published figure contains additional information based on refs 1–3 of the article. In the second paragraph of the article it should have been stated that the apo(*a*) molecule has 37 or more repeats of plasminogen 'kringle IV' not V1.

CONSERVATION BIOLOGY

Divided the fruitflies fall

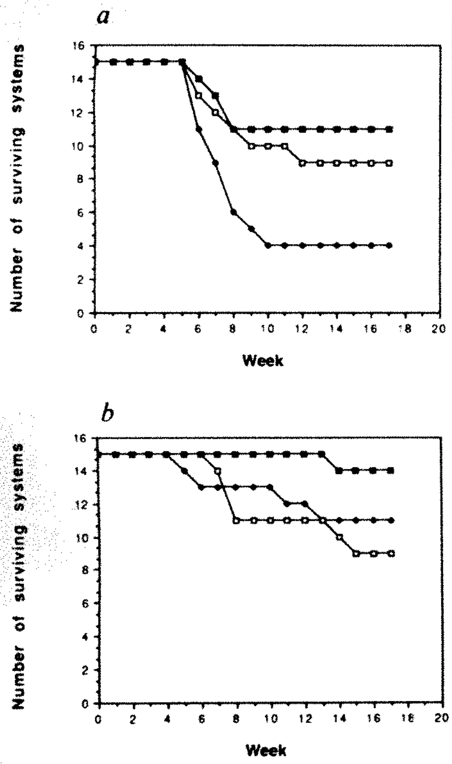
Rolf Anker Ims and Nils C. Stenseth

P 38,673

MAN's destruction of habitat continues to be the leading cause of species extinction[1,2]. It not only reduces the total area of suitable habitats, but also leads to the fragmentation of large continuous populations into smaller, more or less isolated subpopulations. Dynamics within clusters of such subpopulations can be complex[3] because of interactions between numerous factors, such as the degree of subdivision of the subpopulation, the spatial configuration of the habitat fragments and basic population parameters including the rate of increase, dispersal rate, gene flow and social organization. The outcomes of such interactions are very difficult to predict, and such predictions are today almost exclusively derived from simple mathematical models[4]. But Forney and Gilpin[5], in a recent study of two species of fruitfly, *Drosophila hydei* and *D. pseudoobscura*, have reintroduced the use of experimental model systems, whereby populations are manipulated in simple environments in the laboratory. Their observations have serious implications for the general predictions derived from current methods of analysis of population dynamics.

Mathematical models for predicting population dynamics have been criticized for being too simplistic and inconsistent, often disregarding essential population processes[4,6]. This criticism is illustrated by the current debate over whether single large reserves or several small reserves are the most effective[7,8]. Mathematical models present a dilemma: simplistic models have the advantage of being open to thorough analysis but are intrinsically general in nature, whereas complex models that reflect reality more accurately, by way of assumed processes, cannot be analysed thoroughly. The alternative approach, using experimental model systems, strives also to predict population changes from habitat fragmentation by isolating and manipulating the set of factors presumed to be most important. Experimental systems are generally more realistic, and more suited to the study of behavioural responses of organisms to the entirely new conditions created by man. They can, in fact, provide both predictions about real-world phenomena and tests of mathematical models.

In their new study, Forney and Gilpin have compared the fate of *Drosophila* in



The number of surviving replicates in the different experimental systems as a function of time for *a*, *D. pseudoobscura* and *b*, *D. hudei*. ◆, Separated system; □, connected system; ■, large system.

three environments: one in which the original population is divided in two, each subpopulation being confined to a separate culture bottle; a similar system in which the bottles are connected by a corridor that allows migration at the rate of one fly each generation; and a large unified system, in which the initial population and total habitat were twice that of the other systems. Each system, replicated 15 times for both species, was run for 6–8 generations.

Although the total volume of habitat and the total amount of food provided were expected to facilitate subpopulation sizes larger than 20–50 (regarded as the minimum viable size for populations[6]), many of the populations became extinct. Extinctions were, however, less common in the larger system, a twofold increase in population space yielding a sixfold increase in the mean time to extinction. In the separated system large density fluctuations sometimes caused the isolated subpopulations to fall below the minimum viable size, resulting in extinctions due to demographic stochasticity.

Interestingly, the two *Drosophila* species responded differently to the experiment (see figure). *D. hudei* survived better than *D. pseudoobscura* in large and separated systems owing to its higher average population density. *D. pseudoobscura*, on the other hand, survived better in the connected system than in the separated system, and better than *D. hudei* in the connected system, because of a higher dispersal ability, which tended to dampen the population fluctuations within subpopulations.

Forney and Gilpin conclude, somewhat unsurprisingly, that large, continuous populations are more viable than divided ones. Migration corridors, however, do not seem to compensate for this difference[9], a finding of particular relevance to the debate over whether such corridors are effective in maintaining populations in conservation areas. Furthermore, the very different response by two closely related species questions the generality of predictions derived from mathematical models.

The robustness of these results can only be assessed when their experimental design has been replicated for other experimental model systems and under different conditions. Future experiments performed under simplified laboratory environments should be complemented by experiments under more complex and realistic, seminatural field conditions. One such experiment, using an insect predator–prey system, has already been published[10]. Our model experiments and those of M. Gaines and R. Holt (University of Kansas) are of the same kind as those by Forney and Gilpin, but use small mammals in manipulated landscapes.

Relative to studies on natural populations, experimental systems are inexpensive in terms of time, labour and money. This facilitates adequate replication and consequently high statistical significance of the results. Of course, experimental model systems can never replace studies on natural systems, but they may aid in the design of large-scale field projects which thereby could more easily focus on the most significant of the 'jungle' of processes operating in any natural system. Model systems can also provide the spatial and temporal scales[11] required for studies on such natural systems. But, more importantly, experimental model systems may provide an insight of profound value to conservation biologists fighting against the rapidly growing number of species heading for extinction and may indeed be an important complementary approach to the conventional methods of population analysis. □

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1. *Red Data Book* (The IUCN Conservation Monitoring Centre, Cambridge, 1988).
2. Brown, J. & Roughgarden, J. *Science* (in the press).
3. May, R. *Ecology* **67**, 1115–1126 (1986).
4. Simberloff, D. *A. Rev. Ecol. Syst.* **19**, 473–511 (1988).
5. Forney, K.A. & Gilpin, M.E. *Conserv. Biol.* **3**, 45–51 (1989).
6. Lande, R. *Science* **241**, 1455–1460 (1988).
7. Higgs, A.J. & Usher, M.B. *Nature* **285**, 568–569 (1980).
8. Burkey, T.V. *Oikos* **55**, 75–81 (1989).
9. Simberloff, D. & Cox, J. *Conserv. Biol.* **1**, 63–71 (1987).
10. Kareiva, P. *Nature* **326**, 388–390 (1987).
11. Wiens, J.A., Addicott, J.F., Case, T.J. & Diamond, J. in *Community Ecology* (eds Diamond, J. & Case, T.J.) 145–153 (Harper & Row, New York, 1986).

DAEDALUS

Flash and bang

Conventional explosives are dangerously powerful. They have to be, as each small volume of explosive must release enough shock energy to fire the adjacent volume: that is how an explosion propagates. But Daedalus points out that some explosives escape this limitation. A mixture of hydrogen and chlorine, for example, is photosensitive. An intense flash of light can shine through the whole volume and set it all off without any shock having to traverse it. DREADCO's chemists are now exploiting this principle to create new, safe, feeble explosives.

They are synthesizing nitro-substituted polymer-chain molecules with a photosensitive 'detonator' group at one end of the chain. When exposed to the right wavelength of light, the detonator photodissociates, and a radical decomposition explosion propagates down the chain. The molecular 'gain' (energy released per absorbed photon) can be increased simply by lengthening the chain. A dilute solution of this explosive could never detonate or propagate an explosive shock: its energy density would be too low. But a flash of the right wavelength could fire every photoexplosive molecule in it.

The resulting feeble liquid-phase explosion would heat the solvent to a temperature depending on the initial concentration. Window cleaners, campers and swimming-bath attendants should welcome a harmless powder which dissolves readily in water, and raises it instantly to a chosen temperature when fired by a special flash gun. The sudden expansion would give only a small shock. It should not damage the containing vessel, though the brief molecular agitation might usefully kill suspended bacteria. The disengaged nitrogen and carbon dioxide might also give the water a mild but invigorating fizz.

Dispersed in transparent plastic material, Daedalus's photo-explosive will give a novel photographic film. Exposed in a suitable camera, it will explode only in the bright areas of the image. The sudden local heating and expansion could stamp a metal backing sheet into an instant printing plate. Or it could locally melt the plastic, letting it absorb a backing pigment to create a sort of photographic oil painting.

Daedalus's photo-meltable explosive polymer will also make a wonderful instant glue. The chosen kit of parts need only be clamped together, with strips of plastic photo-explosive in the joints. The DREADCO flash setting gun will beam its light right through the strips in optic fibre fashion. They will melt and expand explosively, impact glueing the joints with molten resin. The whole assembly — table, cupboard or grand piano — will then be firmly bonded in one dramatic flash and bang.

David Jones

The unbegun Big Bang

SIR—While it is most plausible that "the Big Bang is an over-simple view of how the Universe began", as recently stressed by John Maddox[1], it need not be as "philosophically unacceptable" as he contends. My purpose is to dissent from the common but mistaken idea that Big Bang cosmologies imply "that there was an instant at which time literally began and, so, by extension, an instant before which there was no time".

In truth, the so-called 'initial' instant t_0 corresponds to a singularity of the space-time model (as expressed by the Robertson–Walker metric) which has to be taken at face value: because the model is not defined for $t = t_0$, this value does not belong to its physical time domain. In other words, the continuity, or, more to the point, the contiguity of time breaks down. The range of physical time consists only of the open interval $]t_0, \infty[$; the initial time t_0 is not a moment in the life of the Universe, and does not belong to its past — which *a fortiori* cannot extend before t_0. As such, this out-of-reach instant may be said to be infinitely remote, irrespective of its finite numerical value on a conventional timescale. This crucial point has been stressed by Misner[2] who proposes to treat it as "an essential element of cosmological theory" and suggests that we refine our concepts of time accordingly. His plea, however, has been little heard.

This situation is not so new: it is similar to the concepts of limit velocity c, or absolute zero for temperatures T_0, both of which mark true singularities in the relevant theoretical formalisms and can be considered as infinite, that is unreachable, values. Just as we have become accustomed to the apparent paradox of a zero temperature, below which the concept of temperature breaks down, so we should accept the idea of a time origin before which the concept of time makes no sense.

Furthermore, according to the theoretical framework of modern cosmology, namely general relativity, the choice of coordinates used to describe the Universe is arbitrary. Formally, the spatio-temporal parameters through which the Robertson–Walker metric is expressed, may be modified at will. The usual choice of cosmic time, although convenient, is by no means unique. Thus it is possible, through a suitable redefinition of time, to send back the birth of the Universe to (minus) infinity where, or rather *when* it seems to belong. Such a redefinition, already outlined by Misner[2], can be derived through a simple group-theoretical analysis designed to restore the additivity of time, a property lost by the conventional cosmic time[3]. This new, linear time is defined by using the Universe itself as its own clock through its expansion, and leads to a suggestive re-expression of the Robertson–Walker metric. Naturally, on the linear scale, the lifespan of the Universe is infinite, so that never did the Big Bang begin. Thus, one may agree with the title of the article by John Maddox, *Down with the Big Bang*, provided it is understood as 'down in time' (and infinitely so).

Note that no new physics is involved in the introduction and use of linear time rather than cosmic time. Although it might be fruitful for some computational purposes, its main interest is conceptual.

There is certainly a great amount of work to be done for improving or even replacing the standard Big Bang theory as a description of the primitive stages of the Universe. Let us not confuse, however, the form and the meaning of our theories. If the present ones are certainly open to much scientific questioning, their philosophical status does not seem that disquieting — provided that our own understanding raises to match their subtleties. If, as John Maddox foresees, "creationists will have to retreat to the Big Bang", we have not too much to fear, as this retreat will be but an endless one.

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1. Maddox, J. *Nature* **340**, 425 (1989).
2. Misner, C.W. *Phys. Rev.* **186**, 1328 (1969).
3. Lévy-Leblond, J.-M. *Am. J. Phys.* (in the press).

Frozen Neanderthals

SIR—Jared Diamond describes in his News and Views article[1], "The recent spectacular discovery of the first reptile to survive natural freezing". He refers to Storey's 1988 report[2] that hatchlings of the Canadian painted turtle in the laboratory can survive the freezing of 52–53 per cent of total body water at −4 °C and to comparable findings for certain frogs — facts of great significance to their survival in nature. Diamond concludes that "Although the discoveries of the first freeze-tolerant vertebrates seem to bring the dream of thawing out [and resuscitating a frozen Neanderthal] tantalizingly closer . . . they actually push it even further into the realm of science fiction. Only by a series of specialized adaptations lacking in most animals do the painted turtle and the four frog species confine freezing to the extracellular space and so avoid lethal damage".

I agree that obtaining viable frozen–thawed Neanderthals is science fiction — not because they lack specialized adaptations, but rather because to survive millennia, they would have to be maintained at temperatures at which biochemical activity ceases, that is, below −120 °C. Unfortunately, the very act of cooling a whole animal to such temperatures will increase the solute concentrations in its tissues 30-fold or more over normal and will convert essentially all its body water to ice. The result, at least for vertebrates, will be instant death.

The first report of a vertebrate surviving partial freezing to −4 or −5 °C was in 1956, and concerned not a reptile or frog with specialized adaptations but a mammal with no adaptations other than the ability to survive cooling in the absence of freezing to 0 °C and slightly below. In that year Smith and Lovelock[3,4] reported that 30 per cent of golden hamsters survived, without apparent injury, the conversion to ice of 40–50 per cent of their body water as the result of 40–60 min in liquid baths at −5 °C. This result is not dissimilar from those for the painted turtle, except that all four of the latter exhibited no evidence of injury after a 12 h exposure in the partly frozen state to air at −4 °C, at which point half the body water was frozen.

Diamond is correct in emphasizing that survival of whole animals (or of most individual cells) after freezing requires that ice be confined to the extracellular space, but this does not demand that the animal possess special adaptations. Rather, it requires: that the first ice forms with little supercooling, and that it first appears in the extracellular space (which is usually the case); that the subsequent cooling rate is low enough to allow sufficient unfrozen cell water to leave the cells osmotically to keep the chemical potential of the residual cell water close to that of the external ice; or that the temperature does not drop to a level at which ice nucleation of the water in supercooled cells can occur[5]. Specialized adaptations do become important in enhancing the ability of cells and organisms to survive greater quantities of extracellular ice (either by the synthesis of cryoprotectants or by plasma-membrane alterations) or by enhancing their ability to supercool.

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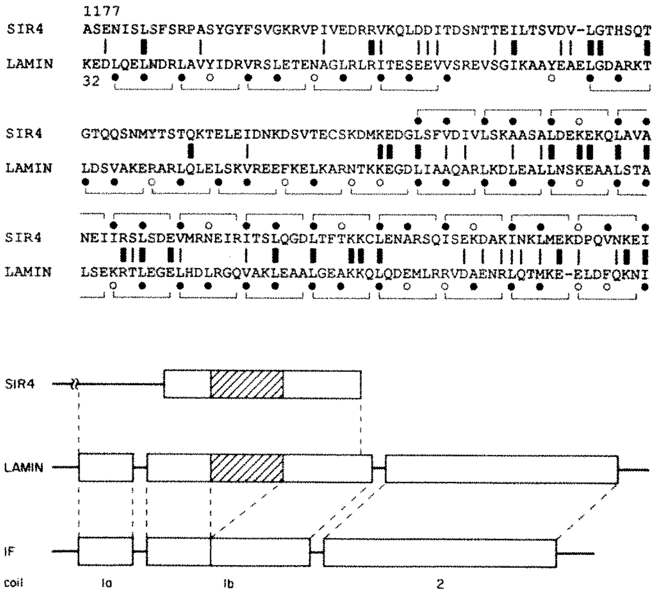
1. Diamond, J.M. *Nature* **339**, 509–510 (1989).
2. Storey, K.B. *et al. Proc. natn. Acad. Sci. U.S.A.* **85**, 8350–8354 (1988).
3. Smith, A.U. *Proc. R. Soc. B* **145**, 407–426 (1956).
4. Lovelock, J.E. & Smith, A.U. *Proc. R. Soc. B* **145**, 427–442 (1956).
5. Mazur, P. *Am. J. Physiol.* **247**, C125–C142 (1984).

Transcriptional silencing and lamins

SIR—The SIR4 protein in the yeast *Saccharomyces cerevisiae* is required for transcriptional silencing of the *HM* mating-type loci and for high mitotic stability of silencer-containing plasmids. As a result of comparing the sequences of proteins involved in DNA replication and transcriptional control, we have noticed that the C terminus of SIR4 (ref. 1) contains 12 in-phase heptad repeats (brackets in upper figure) within a long region of predicted α-helix (rectangles in lower figure). If the amino acids in the heptad repeat are designated (a-b-c-e-f-g)$_n$, residues a and d (circles in the figure) are generally hydrophobic aliphatic amino acids — alanine (A), isoleucine (I), leucine (L), methionine (M), valine (V) — and form the basis for coiled-coil interactions. Of the 24 residues in the a and d positions of the SIR4 heptad repeats, 20 are hydrophobic aliphatic amino acids (closed circles in figure) typical of the heptad repeats found in proteins like myosin, tropomyosin and the intermediate filament (IF) proteins. A computer search[2] revealed that the C terminus of SIR4 is most closely related to the central rod of the human nuclear lamins A and C (23% identity and 36% conservation between residues 123 and 211 of lamins A and C) and that this similarity is not limited to the a and d positions of the heptad repeat. Although lamins A and C are related to other IF proteins[3], the greatest similarity between the C terminus of SIR4 and coil 1b of the lamins A and C lies within a 43-amino-acid region found only in the lamins and not in other members of the IF family (hatched region, residues 120–162 of lamins A and C) suggesting that SIR4 is specifically related to the nuclear lamins.


1177
SIR4 ASENISLSFSRPASYGYFSVGKRVPIVEDRRVKQLDDITDSNTTEILTSVDV-LGTHSQT
LAMIN KEDLQELNDRLAVYIDRVRSLETENAGLRLRITESEEVVSREVSGIKAAYEAELGDARKT
32
SIR4 GTQQSNMYTSTQKTELEIDNKDSVTECSKDMKEDGLSFVDIVLSKAASALDEKEKQLAVA
LAMIN LDSVAKERARLQLELSKVREEFKELKARNTKKEGDLIAAQARLKDLEALLNSKEAALSTA
SIR4 NEIIRSLSDEVMRNEIRITSLQGDLTFTKKCLENARSQISEKDAKINKLMEKDPQVNKEI
LAMIN LSEKRTLEGELHDLRGQVAKLEAALGEAKKQLQDEMLRRVDAENRLQTMKE-ELDFQKNI
SIR4
LAMIN
IF
coil
Ia
Ib
2

Comparison of SIR4, lamin and IF. Identical amino acids are marked by thick bars, conserved amino acids by thin bars.

We note that this region of heptad repeat is an essential functional domain of SIR4 (ref. 1). Furthermore, it is far longer than the 'leucine zipper' found in several transcription factors[4] and the extent and degree of SIR4–lamin similarity is far greater than that noted by Murre *et al.* for the immunoglobin enhancer (kE2) binding protein[5]. This region of SIR4 may serve as a dimerization domain, but it is intriguing to consider that it may also directly interact with the nuclear lamina. In support of this, the REP1 protein encoded by the 2-μM circle plasmid of *Saccharomyces cerevisiae* exhibits a comparable extent and degree of similarity to the lamins and has been shown biochemically to be associated with the karyoskeleton[6]. To explain this, Wu *et al.* postulated that the region of REP1–lamin similarity, found at the C terminus of the REP1 protein, interacts directly with the lamins. It is interesting that the role of REP1 in the mitotic stability of 2-μM plasmids is strikingly similar to the role of SIR4 in the mitotic stability of transcriptional silencer-containing plasmids[7]. In addition to its effect on mitotic stability, however, SIR4 is required for transcriptional silencing by cooperating with silencer-binding proteins[8]. In light of the similarity between SIR4 and the nuclear lamins, it is not unreasonable to suggest that silencing may involve association of the HM loci with the nuclear lamina through SIR4.

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1. Marshall, M., Mahoney, D., Rose, A., Hicks, J.B. & Broach, J.R. *Molec. cell. Biol.* **7**, 4441–4452 (1987).
2. Pearson, W.R. & Lipman, D.J. *Proc. natn. Acad. Sci. U.S.A.* **85**, 2444–2448 (1988).
3. McKeon, F.D., Kirschner, M.W. & Caput, D. *Nature* **319**, 463–468 (1986).
4. Landschultz, W.H., Johnson, P.F. & McKnight, S.L. *Science* **240**, 1759–1764 (1988).
5. Murre, C., McCaw, P.S. & Baltimore, D. *Cell* **56**, 777–783 (1989).
6. Wu, L.-C.,C., Fisher, P.A. & Broach, J.R. *J. biol. Chem.* **262**, 883–891 (1986).
7. Kimmerly, W.J. & Rine, J. *Molec. cell. Biol.* **7**, 4225–4237 (1987).
8. Kimmerly, W.J., Buchman, A., Kornberg, R. & Rine, J. *EMBO J.* **7**, 2241–2253 (1988).

Database pollution

SIR—We have noted that a number of entries in the Genbank, EMBL and NBRF nucleic-acid databases contain vector sequences. We compared three vector sequences (plasmid pBR322, phage M13 and λ-phage) with the sequences in the three databases (GenBank release 59.0, EMBL release 18.0, and NBRF-Nucleic release 34.0) and found that overall there are 62 entries that contain unnecessary vector sequence. Most of these entries (52) contain vector sequences which have no serious consequences (that is, vector sequence was left on the end of a submitted sequence). But 10 of the 62 entries contain vector sequences where the authors have made serious mistakes in interpreting their sequence data. These include, a recent entry on GenBank (accession number M19035) that contains a portion of M13, including part of the polycloning site, in the middle of the presumed protein-coding region. Another two (numbers X05793 and X13410) contain vector sequence within supposed coding regions discussed in the papers and six entries (numbers M10384, M14896, M18076, M21517, V00745 and X12660) that are described as unique sequence are almost entirely vector sequence. These problems would certainly have been avoided if the authors had compared their sequence data with available vector sequences.

In addition to these unwarranted inclusions, there are 9 entries on three databases that contain vector sequences which may be warranted but do not have sufficient documentation.

Inclusion of vector sequence in unique sequence entries adds an unnecessary complication to searching the databases. In some cases it is necessary to include vector sequence in a database entry (such as in fusion constructs), but inclusions of this nature should be clearly annotated. It is not the responsibility of database managers or reviewers to weed out unnecessary sequence information. The responsibility rests firmly with the authors of the sequence.

We have informed GenBank of the sequences in question.

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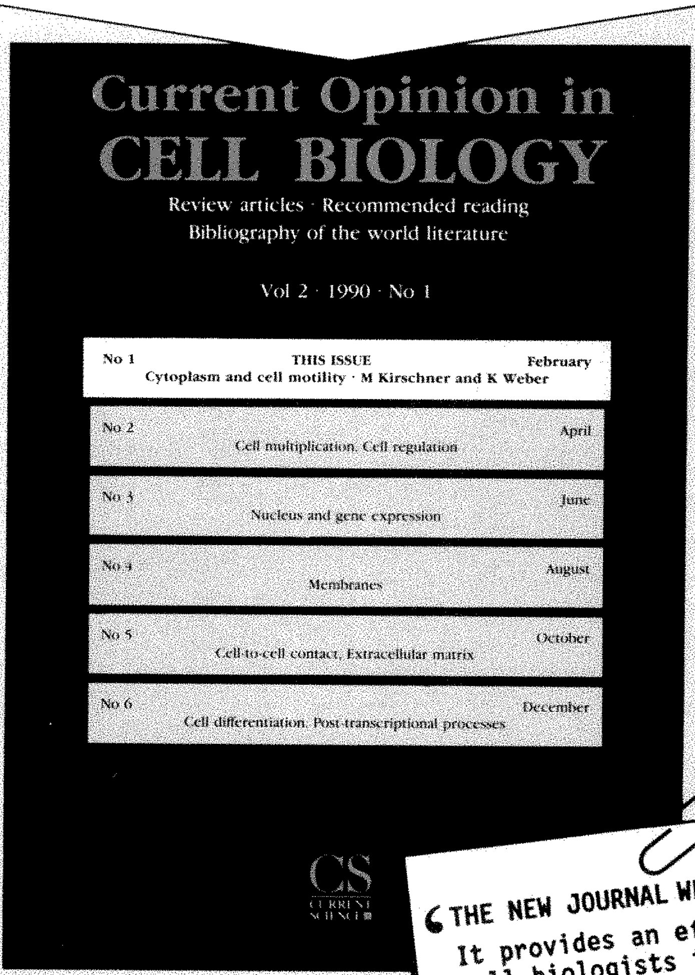

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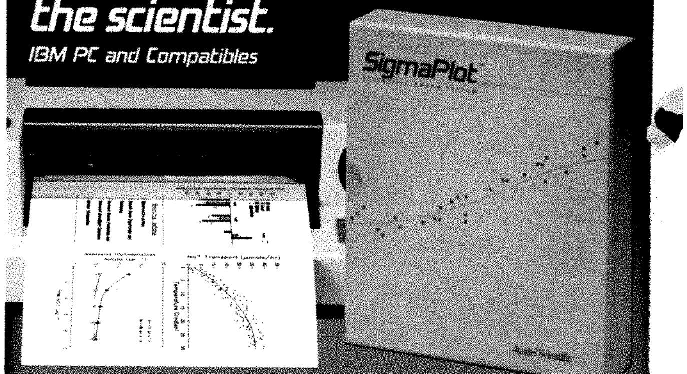

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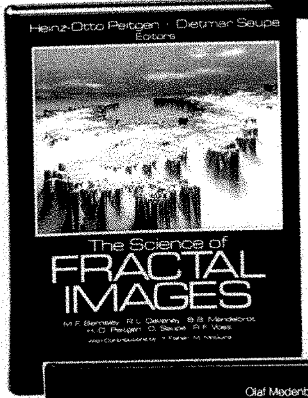

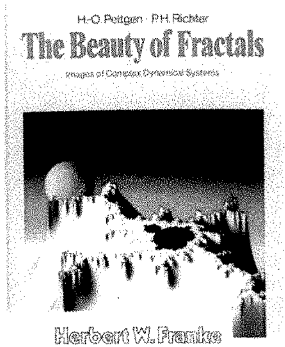
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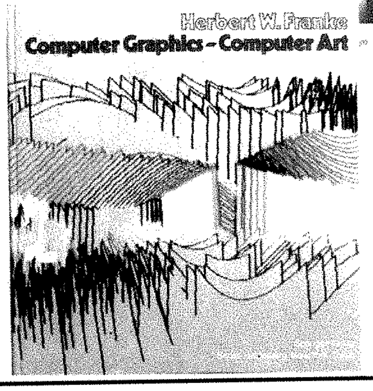

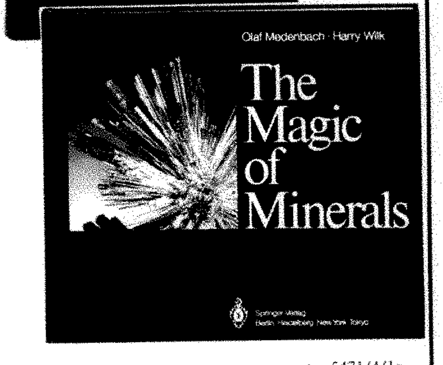

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1989. Approx. 1450 pp. 249 figs. 177 tabs. Hardcover DM 236,–
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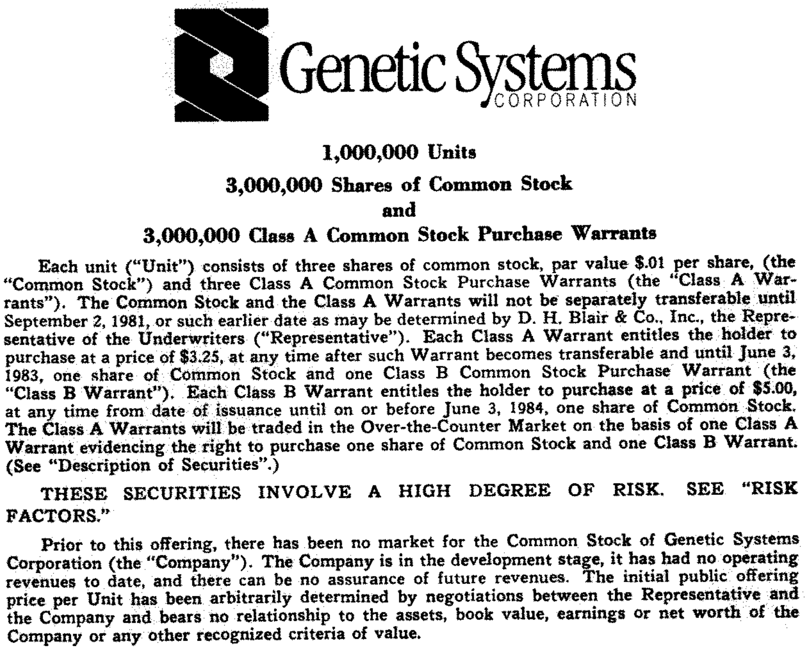
Bulls, bears and bugs

Julian Davies

Gene Dreams: Wall Street, Academia, and the Rise of Biotechnology. By Robert Teitelman. *Basic Books: 1989. Pp.237. $19.95.*

THE rise of modern biotechnology in the 1970s was, with only a few notable exceptions, an American phenomenon. The United States provided a favourable juxtaposition of abundant venture capital and a willingness to take risks, fuelled by the liberal attitude of American universities and their professors. The entire development also rested on the bright young postdocs in molecular biology, who were suddenly provided with a new employment option. Because academic positions were scarce, and conventional industry unattractive, the chance to join like-minded peers in an academic research-style environment under favourable conditions of employment, and without the pressure of grant requests and teaching commitments, was an attractive proposition. The companies themselves were characterized by flexible decision-making, short lines of communication and, above all else, science-based management.



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Risk business — the front page of the prospectus released on 4 June 1981 by D.H. Blair & Co., for the flotation of Genetic Systems.

An important difference between the United States and Europe was that in Europe many people sought security and permanence, while in the United States scientists (and investors) were willing to take a chance. The result was that many of them became multimillionaires. For example, the Blech brothers who featured so strongly in the development of the company Genetic Systems (the leitmotiv of Robert Teitelman's *Gene Dreams*) are in the top ten of the list of biotechnology's rich.

In the book, Teitelman describes well the machinations involved in the setting-up, funding and then, in part, the dismantling of a biotechnology company by illustrating the birth, growth (and death?) of Genetic Systems. The company was born with the same scientific idealism as most of the other biotech companies and, like them, with the same naive approach to business and marketing in the real world of commerce. Robert Nowinski, who was one of the founders, provided most of the scientific driving force both in and out of the company. Much of the Genetic Systems story is built around his ambition and omnipotence, and he is essentially the hero (or scapegoat) of the book.

What I remember best about my years with a biotechnology start-up company (Biogen) was the enthusiasm about the science and the quality of the scientists involved. We hired excellent young PhDs who were willing to change their careers to enter a risky business. There was enormous *esprit de corps*, a biotechnology culture if you will, based partly on the entrepreneurial nature of the business, but also on the fact that we knew that we were involved in tight races with equally talented groups — at Genentech, Cetus and similar companies — to get to a scientific goal and marketable product first. We all knew what the 'products' were. The sense of a race was all the more intense because we were well acquainted with the competition as friends, old academic colleagues or classmates, people with whom we had always had a genial relationship.

Another outstanding feature of the start-up days was the business of financing — the wheeling and dealing to raise private or public funds, the tricks and the enormously hard, and in a sense unsatisfying, task of begging at a high level. We were selling ideas and technology only; it was not until 1983 and 1984 that it became apparent that market-driven and not technology-driven philosophies would be necessary to convince the analysts and investors that we were interested in making a profit. I recall the endless discussions with the big boys (the Abbotts and Hoechsts and Roches) and their apparent conservatism which always heightened our view of ourselves as more innovative, original and better than they were. Even now, scanning the positions vacant columns in *Nature* or *Science*, the ego of young biotechnology companies is obvious. They often begin their advertisements by describing themselves as "leaders", "world-wide leaders", "recognized leaders" or even "recognized world-wide leaders", and they are always "innovative". Modesty is not their strong point. The biotechnology start-ups created an entirely new breed of company, of person and of scientific ideal.

A book which captured all aspects of the exciting and frustrating period, 1975 to 1985, would be an interesting piece of work indeed. *Gene Dreams* is not that book; it is both good and bad. Teitelman is at his best when describing the complex and chicane ways of raising and making money by venture capitalists, and also in his accounts of the internecine relationships and conflicts between scientific founders and would-be commercial managers of new biotechnology companies. The various phases of the foundation and development of Genetic Systems, and how a company born of such high scientific expectations should have become the source of so much manipulation in the hands of its scientific and business management, are described vividly. Indeed, much of what Teitelman recounts of Genetic Systems provides substance to some well-aired criticisms of many of the biotechnology start-ups. The constant changes of strategies and of organization, for example, are painful memories of mine.

An overriding impression that emerges from *Gene Dreams* is of the simultaneous power and incompetence of biotechnology investment analysts. As Teitelman points out, these highly paid Wall Street soothsayers were no better than fortune tellers. It is at the same time amazing and depressing that a brief utterance from a Wall Street analyst could drive a stock price up or down in a matter of hours and make or lose fortunes. How can such lack of knowledge yield such power? Who are the gullible, the investment advisors or the investors themselves? Teitelman presents the capriciousness of this business in an excellent manner.

By contrast, much of the scientific commentary in *Gene Dreams* is plain vacuous. Reading parts of the book to colleagues provoked guffaws of laughter. Was it really necessary to depend so much on metaphor and hyperbole? Do oncogenes have "complex splendor"? What is the relationship between a small molecule conjugated to an antibody and a marriage bed? (This sounds like a joke question.) Are some cancer cells like soldiers ducking into a fox hole? Did Genetic Systems really hope to build its business on the "fruits" of early 1980s-style promiscuity? And are viruses the Rosencrantz and Guildenstern of cancer research? In what sense is interferon an endlessly fascinating name like a mythical African Kingdom, or more like Velcro than penicillin (because it is a polymer?). I could continue.

In the book, it is repeatedly stressed that biotechnology has not provided the world with a new industrial revolution (apart from its impact on Wall Street). But an important point is missed with respect to the revolution in basic biology produced by genetic engineering. As a result of this technology, we now have a series of biologically important proteins in pure form and in unlimited quantity. Many of these proteins were not even known 10–15 years ago, and they have now become part of many significant studies of the human endocrine, immune and neural systems. The list of cytokines, ligands and receptors grows weekly; synthetic genes encoding these compounds are available commercially (one company currently offers 30 or more). The interleukins reported to date number up to seven and we should be in double figures soon. There *has* been a revolution in fundamental biology as a result of biotechnology — witness, too, our present understanding of the determinants of taste, smell and sight.

THE DICTIONARY OF CELL BIOLOGY

edited by
J.M. Lackie and J.A.T. Dow

December 1989, c.320 pages,
Paper: ISBN:0.12.432561.0, £9.95
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> "The concept we started with is now a reality. Our products represent a fundamental shift in medicine, and they're not just working — they're hallmarks."

Reality or hyperbole — Robert Nowinski, founder and provider of much of the scientific driving force behind Genetic Systems, speaks to the shareholders in the 1984 annual report.

Teitelman also insists that the biotech companies have failed when, like Genetic Systems, they sign over their rights (and often more) to large multinationals. Why the crocodile tears? Each of the two parties now has what it did not have before; the one gains financial security, business, organization and marketing services, the other flair, innovation and modern technology. And although he is right in saying that, in business terms, the biotechnology industry has not come up to expectations, that begs the question of who raised the expectations in the first place. The answer, I fear, is mostly the public relations people.

Gene Dreams is undoubtedly a good read. It is relatively free of errors (although I take exception to the suggestion that the word 'biotechnology' was invented on Wall Street) and gives a realistic presentation of what has happened, and is happening, in the venture-capital field with respect to the attempted development of an industry based on biotechnology. Teitelman also provides an accurate picture of the internal and external conflicts affecting biotech companies, and the larger-than-life personalities involved. But this book does not do for its subject what Horace Judson's *Eighth Day of Creation* did for the molecular biology of the 1950s–1970s. For that, someone such as Judson with a more subtle journalistic style, and the ability to capture the scientific excitement as well as the business intrigues, will be needed. There is a cracking story to be told, so why over-embellish and sensationalize it? ☐

Julian Davies is in the Biotechnology Department, Institut Pasteur, 28 rue du Dr Roux, 75724 Paris Cedex 15, France.

DNA in flux

J.R.S. Fincham

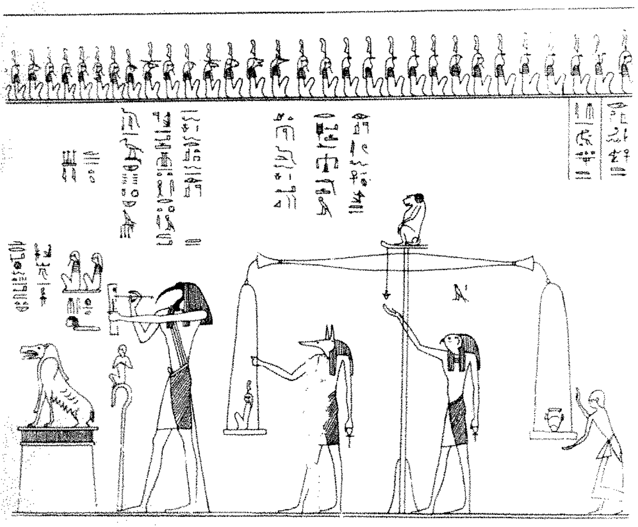
Mobile DNA. Edited by Douglas E. Berg and Martha M. Howe. *American Society for Microbiology, 1913 I Street, NW, Washington, DC 20006: 1989. Pp.972. $75 (ASM member), $95 (non-member).*

AT THE level of the DNA sequence, chromosome structure is in a state of constant flux, usually slow but sometimes surprisingly rapid. Many processes subversive of conventional genetics have evidently been going on for aeons in the natural world, and over the past several decades descriptions of them have greatly proliferated in the scientific literature. The last attempt to pull the whole complex of phenomena together in a single volume was in 1983, when J. Shapiro edited the monograph *Mobile Genetic Elements* for Academic Press. Now Berg and Howe, supported by the American Society for Microbiologists, have provided us with a more than adequate update.

The 43 chapters by different authors cover several very different kinds of genomic change. First there are the well-controlled DNA rearrangements through which organisms of various kinds can create adaptive diversity from a common initial cell type. These include, in vertebrates, the differentiation of cells of the immune system (only immunoglobulin heavy-chain-class switching is dealt with here) and many examples of DNA-based clonal diversification in bacteria and protozoa.

The second kind of DNA mobility is due to the activities of parasites, and includes the integration and excision of the genomes of temperate bacteriophages such as phage-λ and Mu-1, the insertion of reverse transcripts of retrovirus genomes into the chromosomes of animals and plants, and the so far isolated case of insertion of a plasmid DNA segment into plant chromosomes as part of the infection strategy of *Agrobacterium tumefaciens*. From the point of view of the parasite, if not of the host, these processes are precisely controlled and clearly adaptive.

We enter a much more problematical area when we come to the great range of repetitious DNA sequences, present to a greater or lesser extent in virtually all organisms, that have been described, always in derogatory terms, as 'selfish', 'ignorant' or 'junk'. What they all have in common is the capacity to appear at new sites in the genome, though the frequency with which they move varies enormously. Some of them look like dead or crippled retrovirus genomes or, like the retrovirus-related elements best studied in yeast and *Drosophila*, seem to have settled to the role of benign molecular parasites, no



Matters of the heart — Ancient Egyptians believed the heart to be the seat of thought, emotion and intelligence — essential to both the living and dead. This reproduction of a twelfth-century BC papyrus shows a heart from the dead being weighed against a feather which represented law, truth and order. Entry to the afterlife was allowed only if the feather outweighed the heart. Taken from *The Medical Skills of Ancient Egypt* by J. Worth Estes, published by Watson.

longer forming infective particles and only occasionally moving to new chromosomal sites through transcription into RNA and reverse-transcription back to DNA. Others, like the IS elements and transposons of bacteria, the P element of *Drosophila* and the numerous transposable elements now identified in maize and antirrhinum, seem to move quite actively by excision and reinsertion without an RNA intermediate. They resemble the retrovirus-related elements only in encoding the enzymic apparatus for their own movement. Whether the so-called LINES (long interspersed sequences), so abundant in mice and men, are self-moving remains a moot point — they have long open-reading-frames that could code for something with a rather remote resemblance to a reverse transcriptase.

Beyond these there is a host of apparently 'ignorant' sequences which are strongly suspected of having been amplified and dispersed willy-nilly by virtue of reverse transcriptase activity provided by something outside themselves. These elements include pseudogenes (excluded from this volume, presumably because they have no ability to move again) and a great host of SINES (short interspersed sequences) that evidently derive from abundant small RNA molecules transcribed by RNA polymerase 3. SINES are thought to be capable, at least until they decay by mutation, of multiple rounds of proliferation because they carry their own transcriptional promoters with them when they move.

Berg and Howe's compilation extends somewhat beyond these topics to include chapters on illegitimate recombination of DNA in prokaryotes and eukaryotes. M. Meuth's article on eukaryotes is particularly interesting but, surprisingly, does not address the question of how cells of higher eukaryotes are apparently able to integrate into their chromosomes any random bit of non-homologous DNA that is fed to them. Perhaps there is nothing yet to be said on this strange property, on which nearly all current transgenesis experiments depend.

Given that the net was cast so wide, it is perhaps a pity that certain other topics peripheral to the main theme were not included. For example, the tandemly repetitious species-specific 'satellite' DNA arrays found particularly in heterochromatin must certainly have resulted from amplification and dispersal relatively recently on an evolutionary time scale. The mechanisms through which this may have happened are at least worth thinking about. There could be a connection with the spectacular specific gene or chromosome-segment amplification that mammalian cells are capable of under special conditions of selection or stress. Such amplification arguably deserved more than the brief mention and diagram that it gets in Meuth's article.

All of the chapters are by authors with deep experience of their respective subjects. Some, for example Varmus and Brown's 56-page article on retroviruses, with its 397 references, might have been marketable as admirably concise mini-monographs in their own right. The general layout of the book is attractive and clear, much in the style of *Microbiological Reviews*, with a detailed contents page preceding each chapter and plenty of headings and subheadings to help one find particular bits of information.

My only serious reservation about this authoritative and useful volume is that no attempt has been made, either implicitly in the arrangements of the chapters, or explicitly in the form of a general introduction or summary, to provide a scheme of classification of the very diverse phenomena described. Taxonomy may or may not represent real phylogenetic or mechanistic relationships, but at least it helps one to get a mental grip on what may otherwise seem a maze of phenomena. ☐

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Between Tethys and Pacific

A.M.C. Şengör

Geological Evolution of South-East Asia. By Charles S. Hutchison. *Clarendon: 1989. Pp. 368. £65, $135.*

Geological Evolution of South-East Asia forms a connecting link between the two previous volumes in the *Oxford Monographs on Geology and Geophysics* (*The Geology of China*, published in 1986, and *Phanerozoic Earth History of Australia*, 1984). It is a most welcome addition to the series, for it deals with an area about which no modern comprehensive regional treatise exists.

Hutchison's text consists of nine principal chapters arrayed in an unusual order. Following a seven-page introduction, which is devoted mainly to a review of the history of geological studies in the individual countries covered in the book, Hutchison goes on to describe the familiar late Mesozoic and Cenozoic tectonic features such as island arcs, back-arc basins, small oceans, microcontinents, and the main fracture systems of South-East Asia. Chapter 3 is reserved for a description of the Cenozoic sedimentary basins, and here I applaud the author's view that "it is preferable to use terminologies related to the elements of plate tectonics". He erects 12 categories of basin, representing divergent, convergent and intra-plate environments. A curious omission here is strike–slip related basins and some of the more prominent of such basins in South-East Asia are hidden under Hutchison's only unfortunate category, that derived from an influential but misleading basin classification, the uninformative "Pannonian-type".

Chapter 4, "Phanerozoic Tectonic Framework", is an introduction to and a synopsis of the following five chapters. Hutchison defines the sutures and the suture-bound blocks in the region and gives a review of the tectonic evolution, much of which seems to be based on Y.G. Gatinskiy's doctoral thesis published in Russian in 1986 and to have inherited its schematism. Although Hutchison pays lip service to terranology ("the region represents an assemblage of terrains"), he wisely avoids the much-ventilated 'terrane analysis' and sticks to describing the suture-bound blocks of both Cathaysia and Gondwana-Land affinities, plus the intervening sutures together with their ophiolites. Finally, Chapters 8 and 9 cover "The Great Sunda-Pacific Volcanic Arcs", and Hutchison's long-term love, "Granite and Associated Plutonic Rocks".

I have mostly good things to say about this book. In the preface, the author candidly states that

> This is not a standard regional geology, and the selection of the title allowed me to emphasize tectonic models, but at the same time to document the stratigraphy and igneous events. I have always taught my students that every aspect of geology involves interpretation . . . . This book is, therefore, a personal interpretation of the region.

In addition, it contains much information on Tertiary oil and gas basins, but "Unfortunately, mineral deposits [Hutchison's speciality] have been excluded due to length constraints". Hutchison is meticulous in his documentation and conscientious in his presentation. I disagree with a number of his interpretations, but am happy to see that he almost always mentions the alternatives and gives references to them.

The 30-page reference list at the end of the book is an invaluable introduction to the scattered sources of information in South-East Asia, and some of the few critical omissions (for example, the 1986 thesis of N. Sikumbang on late Cretaceous obduction in the Meratus Mountains) are readily excusable given the author's remoteness from large geological libraries. The volume is well produced, and is richly illustrated by line drawings of maps, stratigraphic columns and sections; 17 photographic plates are also included. A drawback here, though, is the paucity of seismic reflection profiles.

All those interested in the geology of South-East Asia, especially its terrestrial parts, or in Tethyan tectonics in general, will benefit from reading Hutchison's book. Because of the tremendous tectonic diversity it documents, it will also be a handy reader for a general course on tectonics at the post-graduate level. ☐

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Growing pains

John de la Mothe

E. W. R. Steacie and Science in Canada.
By M. Christine King. *University of Toronto Press: 1989. Pp.243. $40, £24.*

In 1939 when the photochemist, E. W. R. Steacie, left McGill University to become Director of the National Research Council's (NRC) Chemistry Division, science in Canada was just beginning to become a force to be reckoned with. Until the late 1930s, Canadian science had been in an embryonic state and was far from being world-class. Christine King's intention is to tell how, in the next 20 years, a transformation came about in large part because of Steacie's efforts.

The shallowness of science's roots in Canada can be gauged from the situation in 1918. Then, only 11 doctoral degrees in science had been granted in the entire country; only two or three universities had what could be considered extensive research programmes; and the total value of industrial research and development performed in the country was estimated at less than $135,000 per year. The NRC — which had been founded in 1916 and which had been given the broad mandate of stimulating and coordinating research and development in Canada — had yet to make much of a mark. But what had begun as a one-person operation with an annual budget of $91,600 had grown, by the time of the outbreak of the Second World War, to an institution with a staff of 300, a budget of nearly $1 million and a substantial building of its own. The NRC was to gain further strength during the war and afterwards burgeon into a centre of excellence to which researchers from five continents would gravitate.

The first years of peace were good for Canada. The economy was booming, money was plentiful and the ministers of the Liberal government were sympathetic — even enthusiastic — about building the NRC. The research assistance which the NRC had begun to give to Canadian industry during the war continued with increasingly recognizable (and recognized) effect. Agricultural machinery, mining and transport equipment, construction standards, and pulp and paper processing were all areas which became acknowledged Canadian strengths worldwide.

During this period, industrial research was only one facet of the NRC's activities. With the energetic involvement of Steacie as Director, and later as Vice-President and then (in 1952) as President, the NRC actively promoted the development of a university research system in Canada. Among the obstacles were the antagonism of the big universities to the NRC, as well as the growing sentiment among politicians that the NRC's large budgets should first be questioned, and then, if approved, fed directly into Canada's economic development (and not its ivory towers). In the early 1950s, Steacie negotiated in masterly fashion with the universities and with government officials on three basic propositions: that the universities' duty is to "give society what it needs, rather than what it wants"; that the university is the cornerstone of any nation's research system and capability; and that Canada would suffer a severe shortage of scientific researchers, and run the risk of losing ground economically to other nations, unless something was done.

Steacie's arguments were hard fought but ultimately well received. Of particular importance was his idea of the NRC's postdoctoral fellowships, which would bring scientists to Canada on "princely stipends". As a result of this programme and the NRC's growing research reputation, many researchers not only came to Canada, but stayed, thereby helping to build the university system.

All was not plain sailing, however, either for the NRC or for Steacie. In 1958, the incoming Conservative government immediately began moves to bring the NRC into the civil service. As in Britain, the scientific civil service was a poor relation in terms of both salary and stature. It was a change of policy that was bitterly resisted as it spelt greater management and political accountability for NRC's researchers, and the end of their freedom of scientific enquiry.

In the end, the political pressures won out. But in 1962, when Steacie died of cancer, he had undoubtedly become the greatest figure in Canadian science. In his own modest terms, he had made the NRC one of the main engines of the Canadian research system, and so, as Christine King says, helped "Canada come part of the way along the road from the ox-cart to the bulldozer".

Christine King, who was tragically killed soon after completing the manuscript of this book, attempts to tell the story of a now-powerful institution and of the men who guided it. Above all, her intention is to give some insight into the powerful personality of the NRC's leader at a time when science in Canada was in its formative years. Sad to say she succeeds only in part. Although the book is thoroughly researched and reads well, it fails to convey either a rounded picture of Steacie's character or the actual tensions between science and government that affected his ambitions and directed his choices (and that still exist in Canada today). To some extent, this is the consequence of the style of the book. Scientific biographies such as this tend to portray the protagonist in an heroic light, blurring the background of other personalities and the politics which are an essential part of the overall picture. Nonetheless Dr King's contribution is to recall an important individual at an important time in Canadian history, and for that her book is to be welcomed. ☐

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- Volume 1 in the series *Aperiodicity and Order* was reviewed in *Nature* earlier this year (**338**, 550; 1989). Academic Press has now published the second volume, *Introduction to the Mathematics of Quasicrystals*, which, like the first, is edited by Marko V. Jarić. Price is $59.50, £42.50.

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An analysis of geophysical experiments to test Newton's law of gravity

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Signals reported as evidence for a non-newtonian 'fifth' force at a North Carolina television tower and elsewhere can be explained in a conventional way by postulating small density variations underground. The assumptions employed in earlier analyses which pointed to a failure of the inverse square law are examined and found to be difficult to justify.

THREE recent geophysical experiments to test Newton's law of gravity rely on gravity measurements made along a vertical path: in a 1,000-m deep mineshaft at the Hilton mine, Queensland, Australia[1-3]; on a 600-m television mast, the WTVD tower near Raleigh, North Carolina, USA[4,5]; and in a 2,000-m borehole into the Greenland ice cap, at the Dye-3 radar station[6]. According to classical potential theory, the values obtained on the vertical profile should be exactly predictable from other gravity observations made in the vicinity and, in the case of the underground experiments, knowledge of the density around the shaft. But it has been consistently asserted that prediction and measurement fail to coincide, and this has been taken as strong evidence for the necessity of a small modification to the inverse square law, along the lines called for by a quantum theory of gravity[7] or by introducing a totally new force of nature[8]. The predictions, however, depend on some assumed model of the gravity to interpolate between the survey points. The best calculations so far[5] draw on an unverifiable statistical model. We offer here an alternative test based on the range of permitted densities encountered in the Earth. We apply the test to the television tower experiment, and we show that conventional physics can account for the observations. The same conclusion holds for the Greenland experiment[6]. We are unable to carry out a comparable test for the Australian experiment[2,3] because the original survey data are proprietary; in any case, correction in a mine experiment would require downward continuation, which is intrinsically unstable. We conclude that the present collection of geophysical experiments provides no compelling evidence for discrepancies in the inverse square law of gravity.

Predicting gravity on a vertical profile

The value of the acceleration due to gravity, g, near the Earth's surface is governed by the Earth's rotation and its mass distribution. Traditionally geophysicists correct gravity observations for the Earth's rotation and the attraction of a model Earth; what remains is the sum of the attractions from nearby topography, from density variations in the underlying rock and, for mine and borehole observations, from the surrounding rock. In the case of underground measurements, when the density of the local material is accurately known, its attraction can also be removed. Having been corrected in this way, the gravity field obeys Laplace's equation. According to potential theory[9], the field away from the Earth's surface can then be predicted perfectly if the field is known everywhere on the surface, either by upward continuation for values above the surface, or analytic continuation for those below it.

In practice, exact prediction cannot be made with potential theory: in addition to the inevitable noise in the measurements, one must contend with the need to evaluate an integral that demands knowledge of the field at every point on the surface, not just at the observation sites; different modes of interpolation must lead to different results. Because the prediction from the surface field is inexact, one cannot decide whether or not it is in agreement with the observed value (and so test Newton's law) unless it is accompanied by an estimate of accuracy; this need is particularly acute in the case of downward continuation, which is a well-known example of an ill-posed problem[10,11]. An uncertainty estimate requires a characterization of the gravity field between the measurement sites; without it any gravity value on the vertical path is compatible with every finite surface survey. To see this, imagine expanding the gravity field in a basis of harmonic functions, such as the Fourier-Bessel set used by Romaides *et al.*[5] in their second method of prediction. If enough basis functions are included, one can always find a model field simultaneously in perfect accord with the surface data and the vertical-profile data by incorporating the tower values into the data set and fitting them rather than predicting them. Incidentally, this example illustrates why the prediction of the Fourier-Bessel expansion cannot be equipped with a satisfactory uncertainty estimate: any anomaly can be matched perfectly. Such a procedure, however, runs the risk of generating fields with wild oscillations away from the sample points, but as we shall show, such bad behaviour is not a necessary feature of models satisfying both tower and surface observations in this case.

The first method of analysis of Romaides *et al.*[5] is an example of upward continuation, in which the introduction of an explicit-field model enables the authors to compute uncertainties. They use least-squares collocation (LSC) to predict the values of g along the tower. In LSC, the gravitational field is taken to be the realization of a homogeneous, isotropic statistical process[12]. The estimated power spectrum of the process provides the necessary information about the behaviour of the gravity field between the survey sites. Thus, the predictions of LSC depend not only on the correctness of Newton's law but also on the validity of an elaborate statistical model. As the predictions fail to match the observations, logic dictates that one (or both) of these assumptions must be wrong. To claim that the failure impugns the inverse square law is to regard the statistical model as unassailable. Yet one cannot verify objectively the applicability of a statistical theory of stochastic processes to an object like the Earth's gravity field, which has only one realization. Moreover, it is self-evident that the required spatial homogeneity fails drastically on scales longer than 100 km, as the site is on the boundary of two quite different geological provinces, a major batholith to the north-west and coastal sedimentary sequences to the south-east. Furthermore, as we shall show, there are reasonable (but presumably nonstationary) classical surface fields consistent with the g-values on the tower. We therefore believe that there is no compelling reason to place greater confidence in the statistical model than in the inverse square law.

The second method given in ref. 5 for upward continuation, expansion in a Fourier-Bessel series, cannot be regarded as providing reliable confirmation of their LSC results because, as we mentioned, the uncertainty of its results is uncontrolled.

An alternative test

We propose an alternative strategy, based on the investigation of the field sources—that is, the density distribution in the

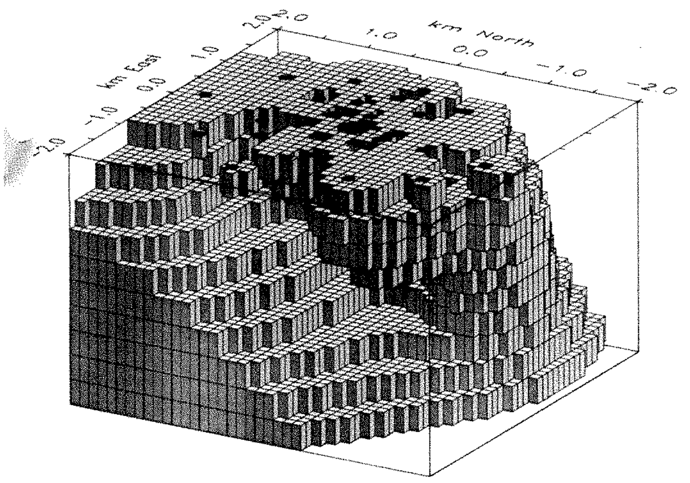

FIG. 3 One possible mass distribution accounting for the Bouguer-corrected data of Fig. 2 and the seven data shown in the topmost curve of Fig. 1. The density of the shaded cells is 0.17 g cm^{-3} less than that of the host rock. The ideal-body inversion fitted 54 gravity observations using 16,000 cells and required 20 min on a Cray XMP computer.

anomalous vertical gradient of 2 mGal km^{-1} reported in ref. 1. If variations are confined to lie below 1,500 m depth, in a volume not subjected to extensive sampling[2], then a density contrast of 0.12 g cm^{-3} is sufficient to produce the observed effect to an accuracy of ~0.05 mGal. The strata of the region show jumps in density that are larger than this by a factor of 2.5 (ref. 2) and hence such a magnitude is not unreasonable. Admittedly, in this calculation we cannot reconcile our model field with the surface survey because the latter is not available; the inclusion of such observations could make a crucial difference. The only published attempt[3] to incorporate that essential information into the analysis has been an estimate of the vertical gradient of g at the surface by means of an integral; values down the shaft and the associated uncertainty estimates have not yet been published.

Application to the tower experiment

Calculation 1. According to ref. 5, the differences between the LSC predictions and the observed values on the tower are due to a small, non-newtonian force. Using ideal-body theory we found the smallest density perturbation required underground to reproduce, by newtonian attraction, the supposedly non-newtonian field. To satisfy the observational constraints, the model produces an attraction that is zero (within 0.005 mGal) at the surface sites of ref. 5 and closely matches the discrepancies on the tower; see Fig. 1. The density contrast needed is only 0.09 g cm^{-3}. In other words, we have found a distribution of mass that accounts for the anomaly on the tower without disturbing the ground observations. It should be understood that this density perturbation is in addition to the density variations implicitly demanded by the LSC analysis. Because of the non-uniqueness of the inverse problem, our model is just one of an infinite-dimensional family of quite different distributions with approximately the same magnitude, each capable of explaining the observations with Newton's law. The fact that the additional density changes are so small makes the demand for new physics unwarranted; had density variations of 1 g cm^{-3} been required, most geologists would have found that improbable.

Calculation 2. In their calculations, Romaides *et al.*[5] upward-continued the observations corrected only for elevation and Earth rotation (the free-air anomalies[18]). Bartlett and Tew[19] point out that short-wavelength gravity signals due to the local topography may not be adequately sampled in the surface gravity survey, with the consequence that the upward-continued field could be biased. In our second calculation, we included corrections for the attraction of the topography (full Bouguer corrections[18]). The removal of the effect of the surface attractions from the observations on the tower gave rise to an important result: the corrected field has an almost exactly constant vertical gradient (Fig. 1). The obvious interpretation of such a field is that it is the natural vertical expression of the regional Bouguer anomaly with its deep origin, free of any controversial aspects whatever. Near geological bodies one expects the horizontal and vertical gravity gradients to be quite similar in magnitude. Horizontal Bouguer gradients in the region (Fig. 2) are typically ~1.5 mGal km^{-1}, caused by the Northeastern Piedmont batholith in contact with the Coastal Plain deposits[20].

We then constructed an underground mass distribution consistent with the fully corrected gravity measurements. An ideal-body model of the Bouger anomaly verifies the simple picture above: we find a large block of material extending to depths of 2.5 km with a density contrast of -0.17 g cm^{-3} (see Fig. 3). The surface observations were fitted to within 0.1 mGal; a larger tolerance must be allowed as additional uncertainties have been introduced with the Bouguer correction from the unknown variation of soil cover thickness and imprecise knowledge of the density of the surface rock. Our model produces an interpolating harmonic field just like the expansion described earlier; evaluation on surface profiles around the tower show the interpolating field to be free of wild oscillations and to lie generally within 1 mGal of the mean regional gradient. This is to be expected considering the low density of the disturbing underground mass.

For computational reasons, both of the ideal-body calculations were limited to a region of ~2 km in radius, centred on the tower. Because of this the attraction of the finite ideal body caused an artificial gravity offset in the data near the tower relative to the distant data; this offset was 1.7 mGal for calculation 1. We found that, because they were so widely spaced, the more distant data could be offset as well by appropriate emplacement of compact masses (of ideal-body density) that had insignificant effects on the gravity values within 2 km. Because such low-density masses can produce a constant offset in the outer data without significantly affecting the inner data, it was unnecessary to include the distant data in the ideal-body calculations.

The more significant result of calculation 2 was not the ideal body itself but the intermediate result needed to obtain it; that is, the correction for terrain effects which removed the curvature in the anomaly. The ideal body in our second calculation provides an illustration of the kind of massive density anomaly required to produce large regional gravity gradients. Including a larger region in the analysis might in fact require a larger density (and would certainly require a larger computing facility) to account for the ground and tower observations, because of the Bouguer anomalies that exist in the region. The results of our first calculation, however, assure us that the increase in density would be only slight.

Conclusions

In summary, we believe that there is no compelling evidence for non-newtonian long-range forces in the three most widely cited geophysical experiments; in two of them, the Greenland experiment and the North Carolina television-mast experiments, we have shown that the gravitational fields of underground density variations with plausible magnitudes can reproduce the effects formerly ascribed to new forces. The Australian experiment has not been subjected to a sufficiently stringent test. It is important to understand that we have not shown the inverse square law to be accurate on the scales of these experiments; we have only shown that the case for its failure has not been established. More sensitive geophysical experiments might still uncover some inadequacy. Two possibilities stand out as particularly worth pursuing: an experiment on a tower that stands on a level sedimentary plane, and a marine experiment in very deep water[21]. □

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1. Stacey, F. D. & Tuck, G. J. *Nature* **292,** 230–232 (1981).
2. Holding S. C. & Tuck, G. J. *Nature* **307,** 714–716 (1984).
3. Stacey, F. D., Tuck, G. J. & Moore, G. I. *J. geophys. Res.* **93,** 10575–10587 (1988).
4. Eckhardt, D. H., Jekeli, C., Lazarewicz, A. R., Romaides, A. J. & Sands, R. W. *Phys. Rev. Lett.* **60,** 2567–2570 (1988).
5. Romaides, A. J., Jekeli, C., Lazarewicz, A. R., Eckhardt, D. H. & Sands, R. W. *J. geophys. Res.* **94,** 1563–1572 (1989).
6. Ander, M. E. *et al. Phys. Rev. Lett.* **62,** 985–988 (1989).
7. Goldman, T., Hughes, R. J. & Nieto, M. M. *Phys. Rev.* D**36,** 1254–1256 (1987).
8. Fischbach, E., Sudarsky, D., Szafer, A., Talmadge, C. & Aronson, S. H. *Phys. Rev. Lett.* **56,** 3–6 (1986).
9. Kellogg, O. D. *Foundations of Potential Theory* (Dover, New York, 1953).
10. Tikhonov, A. N., & Afsenin, V. Y. *Solutions of Ill-posed Problems* (Wiley, New York, 1974).
11. Tikhonov, A. N., Glasko, V. B., Litvinenko, O. K. & Melikhov, V. P. *Akad. Nauk. S.S.S.R. Izv. Phys. solid Earth* **12,** 30–48; English translation 738–747 (1968).
12. Moritz, H. *Advanced Physical Geodesy* (Abacus, Tunbridge Wells, 1980).
13. Parker, R. L. *Geophys. J. R. astr. Soc.* **42,** 315–334 (1975).
14. Ander, M. E. & Huestis, S. P. *Geophysics* **52,** 1265–1278 (1987).
15. Luenberger, D. G. *Optimization by Vector Space Methods* (Wiley, New York, 1969).
16. Gill, P. E., Murray, W. & Wright, M. H. *Practical Optimization* (Academic, New York, 1981).
17. Stark, P. B. & Parker, R. L. *J. geophys. Res.* **92,** 2713–2719 (1987).
18. Garland, G. D. *The Earth's Shape and Gravity* (Pergamon, Oxford, 1977).
19. Bartlett, D. F. & Tew, W. I. *Pap. presented at Fall Meet. Am. geophys. Un.*, San Francisco, December (1989).
20. Grover, D. P. thesis, Univ. North Carolina Chapel Hill (1963).
21. Hildebrand, J. A. *et al. Eos* **69,** 779–780 (1988).

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Primary structure and functional expression of the inositol 1,4,5-trisphosphate-binding protein P_{400}

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Cloning and expression of functional P_{400} protein from cerebellar Purkinje neurons shows that this protein is a receptor for inositol 1,4,5-trisphosphate, a second messenger that mediates the release of intracellular calcium.

THE mouse cerebellum contains five different neuronal cell types. One of these, the Purkinje cell, is considered to play a central role in information processing, because it is the target of all inputs and is the only neuron that sends outputs from the cerebellar cortex. In the course of a study on cerebellar ataxic mutant mice, a membrane glycoprotein P_{400} was found to be enriched in Purkinje cells from normal mice but reduced in the cerebella of Purkinje-cell-deficient mutants[1-3]. The P_{400} protein has a relative molecular mass (M_r) of 250,000 (250K)[1,3] and is phosphorylated[4] in a cyclic AMP-dependent manner[5]. A pulse labelling study with [^{14}C]leucine showed that it is synthesized in the cerebellum[4]. We have solubilized and purified P_{400} protein from mouse cerebella and obtained three independent anti-P_{400} monoclonal antibodies[6,7]. Immunohistochemical study of the cerebellum shows that P_{400} protein is localized to the dendrites and axons, as well as cell bodies of Purkinje cells[7], where immunoreactivity to the protein is localized on the plasma membrane, endoplasmic reticulum (ER) and postsynaptic density of Purkinje cells[7]. Western blot analysis in several other tissues reveals the presence of a small amount of P_{400} protein[6], indicating that it plays an essential part in a general membrane function that is increased in cerebellar Purkinje cells.

Inositol 1,4,5-trisphosphate ($InsP_3$) is known to play an important second messenger role in many cells, including neurons[8]. Recently Supattapone *et al.*[9] purified an $InsP_3$-binding protein (M_r, 260K) from the rat cerebellum. This receptor is a glycoprotein capable of binding to concanavalin A and is a substrate for cAMP-dependent protein kinase[9,10]. They also showed that [^{3}H]$InsP_3$-binding activity is very much higher in the cerebellum than in any other brain area[11,12], and is selectively localized to Purkinje cells[12]. From its characteristics (M_r, glycosylation, cAMP-dependent phosphorylation and tissue concentration), they propose that this receptor protein is identical to the three other cerebellar phosphoproteins—including P_{400}—that have been recently described[9,13,14]. We have also confirmed that P_{400} protein is identical to the $InsP_3$ receptor protein[15].

We report here the cloning of the P_{400} complementary DNA together with its amino-acid sequence deduced from the cDNA sequence[16]. Expression of P_{400} messenger RNA was examined in the cerebellar mutant mice by northern blot analysis and was localized by *in situ* hybridization in cerebellar sections. We demonstrate that NG108-15 cells (mouse neuroblastoma/rat glioma hybrids) transfected with P_{400} cDNA express binding sites for $InsP_3$ and that the predicted structure of the P_{400} protein resembles that of the ryanodine receptor[17], which mediates Ca^{2+} release from the sarcoplasmic reticulum (SR) of skeletal muscle during excitation–contraction coupling.

Cloning of P_{400} cDNA

Two mouse cerebellum cDNA libraries, synthesized by priming with random hexamer or oligo(dT), were constructed in phage-λgt11 expression vectors (Fig. 1). The library was first screened with the three anti-P_{400} monoclonal antibodies (4C11, 10A6 and 18A10)[6,7]. Of 21 immunopositive candidates obtained, five were selected for further study on the basis of their strong immunoreactivity in western blot analysis (data not shown). In northern blot analysis of mouse cerebellar poly(A)$^+$RNA, the cDNA inserts in all five independent clones hybridized to an mRNA of $\sim$10 kilobases (kb), which is long enough to encode the P_{400} protein (data not shown). To obtain more complete clones, we rescreened both the random-primed and oligo(dT)-primed libraries by plaque hybridization with the ^{32}P-labelled cDNA inserts. Two types of clone were obtained; these differed at the polyadenylation sites by 800 bases.

The complete cDNA sequence was determined[16] by sequencing the 12 clones listed in Fig. 1*a*. There is a single large open reading frame[16]. Although the neighbouring sequence (CGGACATGT) of the first ATG shows only modest similarity to the initiation consensus sequence (CCA/GCCATG(G))[18], it is closer to the consensus sequence than the sequence (ACAAAATGT) surrounding the second ATG. The open reading frame continues for 8,247 bases when an in-frame TAG termination codon occurs at nucleotide position 8,248 from the first ATG. This frame was confirmed by sequencing the fusion points between the β-galactosidase gene and cDNA inserts in the five immunopositive clones tested. The sequence of the long type cDNA is 9,871 nucleotides, consisting of a 328 base pairs (bp) 5′ untranslated region, a 8,247 bp coding region and a 1,296 bp 3′ untranslated region which includes a polyadenylation signal (AATAAA) 14 bp upstream of a 23 base poly(A) tract. The short type cDNA is 9,071 nucleotides long, differing only in the 3′ untranslated region (496 nucleotides, including AATAAA 12 bp upstream of a 23-base poly(A) tract). It seems that the long cDNA was derived from the principal transcript, as five out of the seven 3′ clones, primed by oligo(dT), were of the long type.

Structure of the P_{400} protein

On the basis of the cDNA sequence, the P_{400} protein is predicted to comprise 2,749 amino acids and have an M_r of 313K. Although glycosylation is known to contribute to the molecular weight only slightly, this is somewhat greater than the SDS-gel electrophoresis estimate of 250K. This discrepancy may be accounted for by post-translational proteolytic processing, or aberrant electrophoretic migration due to its large size. The amino-acid composition of the predicted polypeptide agrees with that obtained by amino-acid analysis of hydrolysed P_{400} (data not shown). From sequence analysis of immunopositive clones, the amino-acid sequences containing epitopes for the antibodies 4C11, 10A6 and 18A10 are localized from position 679–727, 943–1,237 and 2,648–2,749 (C-terminal end), respectively (Figs 1*b* and 2*a*).

A hydropathy profile of the protein sequence shows the existence of several stretches of hydrophobic amino acids, which could represent multiple membrane-spanning sequences (Fig. 1*b* and 2). Although P_{400} is thought to be an integral membrane protein[6,7], it lacks a definable signal sequence with a hydrophobic stretch at the N-terminus, which indicates that an N-terminal hydrophilic region is exposed on the cytoplasmic face of the membrane[17,19]. By scanning the sequence for hydrophobic stretches[20], nine candidates membrane-spanning regions were detected (a–i in Fig. 2*a*). Preliminary results from an electron microscopy study of metallo-labelled antibodies indicate that the epitopes for 4C11 and 10A6, which recognize the N-terminal region and the central region, respectively, are located to the cytoplasmic side, whereas an 18A10 epitope, which recognizes the C-terminus, is on the luminal side of the ER (N.M. *et al.*, unpublished data). The first two segments (a and b) are, therefore, unlikely to be membrane-spanning. Of the seven remaining segments (c to i), clustering in the C-terminus from amino-acid positions 2,276 to 2,589, all (or some) of the segments presumably form transmembrane α-helices and may traverse either the ER or plasma membranes of cerebellar Purkinje cells an odd number of times (see Fig. 2 legend). Consequently, the large hydrophilic N-terminal domain is exposed on the cytoplasmic face of the membrane, whereas the short C-terminal domain is oriented towards the ER lumen or outside the cell (Fig. 2*b*).

The protein has 21 consensus sequences for *N*-linked glycosylation. Most of these, however, are not involved in glycosylation, because digestion of P_{400} protein with endo-β-*N*-acetylglucosaminidase F causes only small changes in the electrophoretic mobility[6]. Partial digestion of P_{400} by *Staphylococcus aureus* V8 protease generated a polypeptide of M_r ~40K, possessing both binding activity to concanavalin A and immunoreactivity with the C-terminal-specific antibody 18A10 (data not shown). This result suggests that at least the C-terminal region is glycosylated. Additionally, the large cytoplasmic domain contains two sites that could act as targets for serine

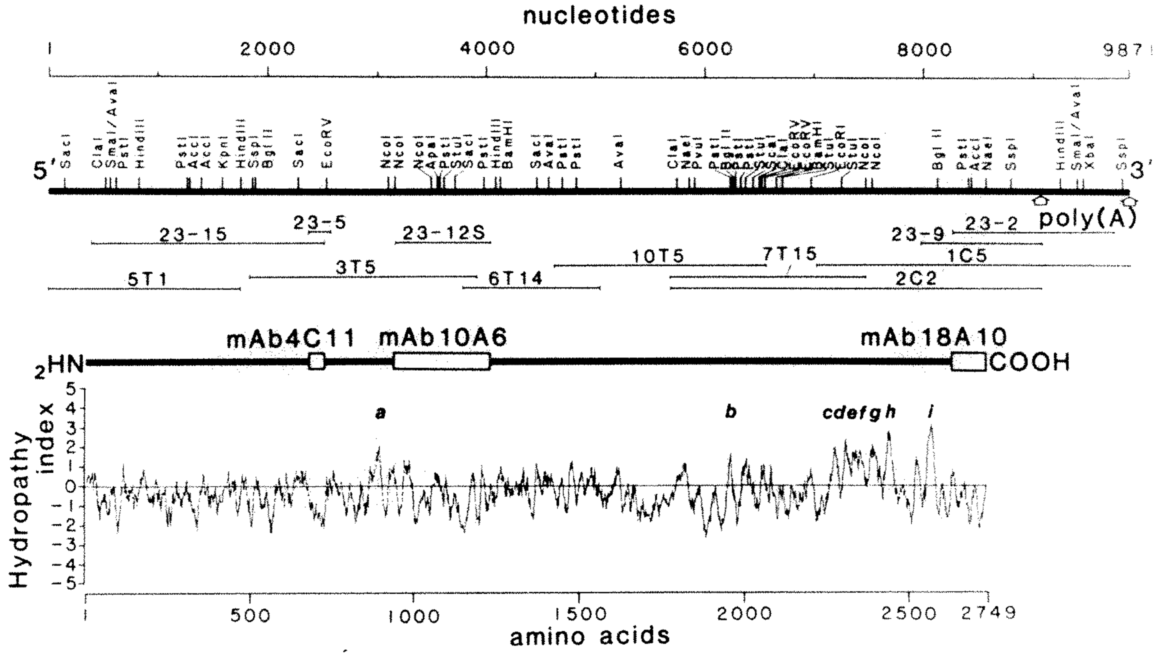

FIG. 1 *a*, Restriction map of the P_{400} cDNA clones. The positions of the 12 overlapping clones are indicated. Two open arrows point to the polyadenylation sites. *b*, Hydropathy plot of protein-coding region. Three open boxes represent the regions containing epitope sequences for monoclonal antibodies (mAb) 4C11, 10A6 and 18A10. Hydrophobic segments (a to i) are indicated.

METHODS. Poly(A)$^+$RNA was isolated from cerebella of two- to three-week-old mice (ICR) by the guanidinium-CsCl method[36], followed by purification by oligo(dT)-cellulose chromatography[36]. The cDNA was synthesized using a random hexamer or oligo$(dT)_{12-18}$ as primers essentially as described previously[37]. For random-primed cDNA synthesis, poly(A)$^+$RNA with a sedimentation coefficient of more than 23S in sucrose gradients (15–30% sucrose gradients spun at 30,000 r.p.m. in a Beckman SW41Ti rotor for 16 h at 25 °C) was used. Both random-primed and oligo(dT)-primed cDNAs were ligated in the lambda-gt11 expression vector[38]. First, the random-primed library was immunoscreened for the production of fusion protein containing putative P_{400} epitopes in the host *E. coli* strain Y1090r$^-$ using monoclonal antibodies[38,6]. At subsequent stages, both the random-primed and oligo(dT)-primed libraries were screened by plaque hybridization using the ^{32}P-labelled cDNA inserts derived from immunopositive clones (clone 23-2, 23-5, 23-9, 23-12S and 23-15 in Fig. 1*a*). The cDNA inserts were subcloned into pUC118 or pUC119 (ref. 39) and sequenced[16] by the dideoxy chain termination method[40]. The hydropathy plot was computed according to Kyte and Doolittle[41]; the window size is 17 residues.

a

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   1 MSDKMSSFLHIGDICSLYAEGSTNGFISTLGLVDDRCVVQPEAGDLNNPPKKFRDCLFKLCPMNRYSAQKQFWKAAKPGANSTTDAVLLNKLHHAADLEK 100
 101 KQNETENRKLLGTVIQYGNVIQLLHLKSNKYLTVNKRLPALLEKNAMRVTLDEAGNEGSWFYIQPFYKLRSIGDSVVIGDKVVLNPVNAGQPLHASSHQL 200
 201 VDNPGCNEVNSVNCNTSWKIVLFMKWSDNKDDILKGGDVVRLFHAEQEKFLTCDEHRKKQHVFLRTTGRQSATSATSSKALWEVEVVQHDPCRGGAGYWN 300
 301 SLFRFKHLATGHYLAAEVDPDFEEECLEFQPSVDPDQDASRSRLRNAQEKMVYSLVSVPEGNDISSIFELDPTTLRGGDSLVPRNSYVRLRHLCTNTWVH 400
 401 STNIPIDKEEEKPVMLKIGTSPLKEDKEAFAIVPVSPAEVRDLDFANDASKVLGSIAGKLEKGTITQNERRSVTKLLEDLVYFVTGGTNSGQDVLEVVFS 500
 501 KPNRERQKLMREQNILKQIFKLLQAPFTDCGDGPMLRLEELGDQRHAPFRHICRLCYRVLRHSQQDYRKNQEYIAKQFGFMQKQIGYDVLAEDTITALLH 600
 601 NNRKLLEKHITAAEIDTFVSLVRKNREPRFLDYLSDLCVSMNKSIPVTQELICKAVLNPTNADILIETKLVLSRFEFEGVSTGENALEAGEDEEEVWLFW 700
                                                                                     ----------------------
 701 RDSNKEIRSKSVRELAQDAKEGQKEDRDILSYYRYQLNLFARMCLDRQYLAINEISGQLDVDLILRCMSDENLPYDLRASFCRLMLHMHVDRDPQEQVTP 800
     ---------------------------
 801 VKYARLWSEIPSEIAIDDYDSSGTSKDEIKERFAQTMEFVEEYLRDVVCQRFPFSDKEKNKLTFEVVNLARNLIYFGFYNFSDLLRLTKILLAILDCVHV 900
                                                                                              ======= a =
 901 TTIFPISKMTKGEENKGSNVMRSIHGVGELMTQVVLRGGGFLPMTPMAAAPEGNVKQAEPEKEDIMVMDTKLKIIEILQFILNVRLDYRISCLLCIFKRE 1000
     =======                                   ----------------------------------------------------------
1001 FDESNSQSSETSSGNSSQEGPSNVPGALDFEHIEEQAEGIFGGSEENTPLDLDDHGGRTFLRVLLHLTMHDYPPLVSGALQLLFRHFSQRQEVLQAFKQV 1100
     ----------------------------------------------------------------------------------------------------
1101 QLLVTSQDVDNYKQIKQDLDQLRSIVEKSELWVYKGQGPDEPMDGASGENEHKKTEEGTSKPLKHESTSSYNYRVVKEILIRLSKLCVQESASVRKSRKQ 1200
     ----------------------------------------------------------------------------------------------------
1201 QQRLLRNMGAHAVVLELLQIPYEKAEDTKMQEIMRLAHEFLQNFCAGNQQNQALLHKHINLFLKPGILEAVTMQHIFMNNFQLCSEINERVVQHFVHCIE 1300
     -------------------------------------
1301 THGRNVQYIKFLQTIVKAEGKFIKKCQDMVMAELVNSGEDVLVFYNDRASFQTLIQMMRSERDRMDENSPLMYHIHLVELLAVCTEGKNVYTEIKCNSLL 1400
1401 PLDDIVRVVTHEDCIPEVKIAYINFLNHCYVDTEVEMKEIYTSNHMWKLFENFLVDICRACNNTSDRKHADSILEKYVTEIVMSIVTTFFSSPFSDQSTT 1500
1501 LQTRQPVFVQLLQGVFRVYHCNWLMPSQKASVESCIRVLSDVAKSRAIAIPVDLDSQVNNLFLKSHNIVQKTALNWRLSARNAARRDSVLAASRDYRNII 1600
                                                                                             ▲
1601 ERLQDIVSALEDRLRPLVQAELSVLVDVLHRPELLFPENTDARRKCESGGFICKLIKHTKQLLEENEEKLCIKVLQTLREMMTKDRGYGEKQISIDESEN 1700
1701 AELPQAPEAENSTEQELEPSPPLRQLEDHKRGEALRQILVNRYYGNIRPSGRRESLTSFGNGPLSPGGPSKPGGGGGGPGSSSTSRGEMSLAEVQCHLDK 1800
                                                           ▲
1801 EGASNLVIDLIMNASSDRVFHESILLAIALLEGGNTTIQHSFFCRLTEDKKSEKFFKVFYDRMKVAQQEIKATVTVNTSDLGNKKKDDEVDRDAPSRKKA 1900
1901 KEPTTQITEEVRDQLLEASAATRKAFTTFRREADPDDHYQSGEGTQATTDKAKDDLEMSAVITIMQPILRFLQLLCENHNRDLQNFLRCQNNKTNYNLVC 2000
                                                                ======= b =======
2001 ETLQFLDCICGSTTGGLGLLGLYINEKNVALINQTLESLTEYCQGPCHENQNCIATHESNGIDIITALILNDINPLGKKRMDLVLELKNNASKLLLAIME 2100
2101 SRHDSENAERILYNMRPKELVEVIKKAYMQGEVEFEDGENGEDGAASPRNVGHNIYILAHQLARHNKELQTMLKPGGQVDGDEALEFYAKHTAQIEIVRL 2200
2201 DRTMEQIVFPVPSICEFLTKESKLRIYYTTERDEQGSKINDFFLRSEDLFNEMNWQKKLRAQPVLYWCARNMSFWSSISFNLAVLMNLLVAFFYPFKGVR 2300
                   △                                                            ======== c ========
2301 GGTLEPHWSGLLWTAMLISLAIVIALPKPHGIRALIASTILRLIFSVGLQPTLFLLGAFNVCNKIIFLMSFVGNCGTFTRGYRAMVLDVEFLYHLLYLLI 2400
            ========= d =======       ======= e ======= ========= f =========                  ======= g
2401 CAMGLFVHEFFYSLLLFDLVYREETLLNVIKSVTRNGRSIILTAVLALILVYLFSIVGYLFFKDDFILEVDRLPNETAVPETGESLANDFLYSDVCRVET 2500
     =======                                =========== h =========
2501 GENCTSPAPKEELLPAEETEQDKEHTCETLLMCIVTVLSHGLRSGGGVGDVLRKPSKEEPLFAARVIYDLLFFFMVIIIVLNLIFGVIIDTFADLRSEKQ 2600
                                                                          ========== i ========
2601 KKEEILKTTCFICGLERDKFDNKTVTFEEHIKEEHNMWHYLCFIVLVKVKDSTEYTGPESYVAEMIRERNLDWFLRMRAMSLVSSDSEGEQNELRNLQEK 2700
                                                    -----------------------------------------------------
2701 LESTMKLVTNLSGQLSELKDQMTEQRKQKQRIGLLGHPPHMNVNPQQPA
     -------------------------------------------------                                                   2749
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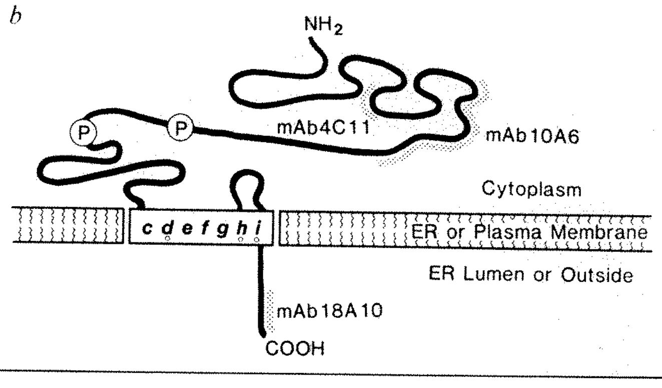


◀ FIG. 2 Predicted amino-acid sequence and structural model of P_{400} protein. *a*, The amino-acid sequence of P_{400} protein was deduced from its cDNA sequence[16] (EMBL database accession number X15373). The regions containing epitope sequences for monoclonal antibodies are shown by dashed lines. The proposed membrane-spanning sequences are represented by double dashed lines (a–i). The filled arrowheads mark the potential phosphorylation sites for cAMP-dependent protein kinase. The 3′-deletion construct shown in Fig. 5 encodes a polypeptide from the first methionine to the cysteine of position 2,215, marked with the open arrowhead. *b*, A schematic model for the topology of P_{400} protein in the membrane. A computer program (ALOM), for prediction of membrane-spanning segments[20], in the Integrated Database and Extended Analysis System (IDEAS) was used through the National Institute of Genetics (NIG) in Japan. Possibilities for membrane-spanning become confident in the following orders: segments i > h > d > g > a > c > f > e > b (peripheral:integral odds = 7.0E-07 > 8.1E-06 > 5.1E-04 > 4.5E-03 > 1.2E-02 > 3.2E-02 > 7.8E-02 > 4.0E-01 > 5.3E-01, respectively). To orient termini to opposite sides, the P_{400} protein should traverse the membrane an odd number of times. Epitopes for antibodies 4C11 and 10A6 are localized to the cytoplasmic side, indicating that segment a is not membrane-spanning. We assume, therefore, that P_{400} traverses the membrane at most seven times (c to i) and at least 3 times because of the presence of three reliable segments d, h and i with high odds. Based on this prediction and the epitope localizations, we have designed the structural model for P_{400} as a membrane-spanning protein. Putative transmembrane domains are indicated by an open box. The C-terminal domain is either located in the ER lumen or outside the cell by anchoring to the ER and plasma membrane, respectively. The regions containing epitope sequences for monoclonal antibodies are shown by dotted lines. The potential phosphorylation sites are represented by an encircled P.

phosphorylation (amino acids 1,588 and 1,755), a possibility supported by the fact that a serine residue of P_{400} protein is known to be phosphorylated in a cAMP-dependent manner[5].

P_{400} expression in Purkinje cells

In situ hybridization histochemistry clearly shows that P_{400} mRNA is localized in the perikaryon of Purkinje cells of the cerebellum (Fig. 3). We examined gene expression of P_{400} in the cerebellar ataxic mice by northern blot analysis (Fig. 4). The *staggerer* mutant Purkinje cells have a withered dendritic arbor lacking the tertiary branchlet spines that serve as the sites of synapse between the granule cells and Purkinje cells[21]. The disorder of Purkinje cells is most severe in the *Purkinje cell degeneration* (*pcd*) and *nervous* mutants, where most of these cells degenerate selectively (95–99% in *pcd*[22] and about 85% in *nervous*[23]). The cerebella of these Purkinje cell-deficient mutants contain P_{400} mRNA at extremely low levels, as expected (lanes 2, 3 and 4 in Fig. 4). On the other hand, the cerebellum of the *weaver* mutant, which lacks granule cells with no significant loss of other cerebellar neurons[24], contains an increased amount of P_{400} mRNA compared with the wild-type cerebellum (lane 4). This increase in the *weaver* mutant was also observed at the protein level[1,4] and is caused by a relative increase in the proportion of Purkinje cells in the cerebellum, due to the dearth of granule cells.

These results clearly indicate a predominant distribution of P_{400} gene expression in the cerebellar Purkinje cells. Our preliminary experiments, however, have shown that the expression of P_{400} mRNA can be detected at a level of about one fiftieth in the cerebrum and at much lower levels in other tissues (data not shown), indicating that P_{400} expression is widespread and possibly general.

$InsP_3$-binding properties

Our recent results indicate that P_{400} protein has $InsP_3$-binding activity[15]. To examine whether the P_{400} cDNA clone encodes an $InsP_3$-binding protein, we performed $InsP_3$-binding assays on protein expressed in the neuroblastoma/glioma hybrid cell line NG108-15. In the neuron-like NG108-15 cells, it is known that $InsP_3$ acts as a second messenger and transiently raises intracellular Ca^{2+} (ref. 25). This cell contains an endogenous protein immunoreactive to the anti-P_{400} monoclonal antibody 4C11 (Fig. 5*a*, lane 4). This protein cross-reacts equally with all three of our monoclonal antibodies (data not shown). It is interesting that the molecular size of the endogenous P_{400} protein

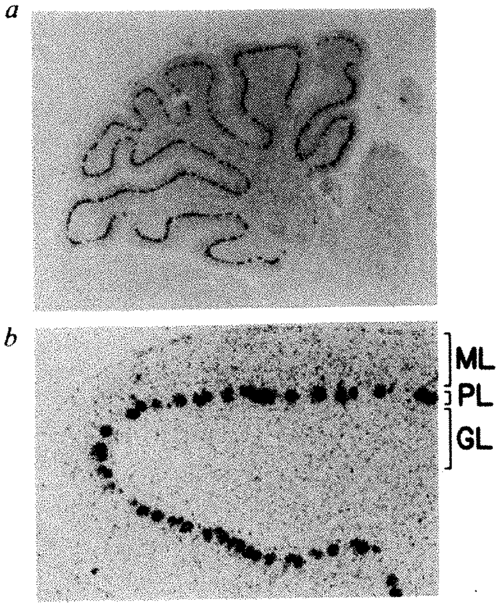


FIG. 3 Localization of the P_{400}-specific mRNA by *in situ* hybridization. *a*, Autoradiograph of a sagittal section of the cerebellum. *b*, Higher magnification of *a*. ML, molecular layer; PL, Purkinje cell layer; GL, granular layer. Sections (6 μm) in paraffin were prepared from the cerebellum of 30-day-old ICR mice. *In situ* hybridization with a random-primed ^{35}S-labelled P_{400} cDNA was as before[42].

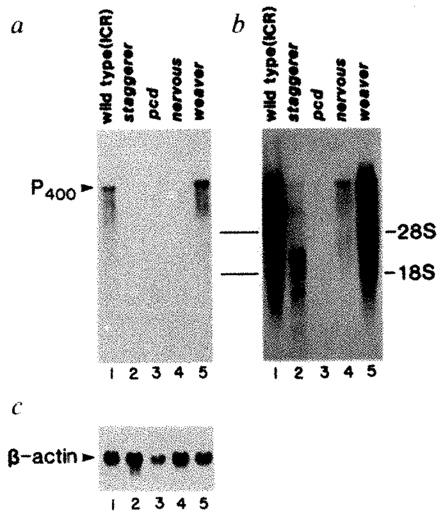


FIG. 4 Northern blot analysis of P_{400} mRNA in the cerebella of mutant mice. *a*, Wild-type ICR, lane 1; *staggerer*, lane 2; *pcd*, lane 3; *nervous*, lane 4; and *weaver*, lane 5 mice. *b*, Long exposure of the hybridization membrane in *a*. *c*, Rehybridization of the same blot in *a* with a β-actin probe to assess the relative amounts of RNA in each lane. Although an equal amount of total RNA was used, *pcd* showed a weaker signal for β-actin mRNA.

METHODS. Total cellular RNA (20 μg) from the cerebella was electrophoresed on a 1% agarose-formaldehyde gel[36], blotted to a nylon membrane (Biodyne. A, Pall) and hybridized at 42 °C for 20 h with random-primed ^{32}P-labelled P_{400} cDNA or β-actin cDNA probes (1×10^9 c.p.m. μg^{-1}, $1\text{-}2 \times 10^6$ c.p.m. ml^{-1}) in 50% formamide, 5 × Denhardt's solution, 5 × SSC, 50 mM sodium phosphate (pH 6.5), 0.1% SDS, 100 μg ml^{-1} heat-denatured herring sperm DNA and 10% dextran sulphate. The membrane was washed twice in 2 × SSC and 0.1% SDS at room temperature for 10 min, twice in 0.1 × SSC and 0.1% SDS at 65 °C for 30 min. The blot was exposed at −70 °C to X-ray film with an intensifying screen.

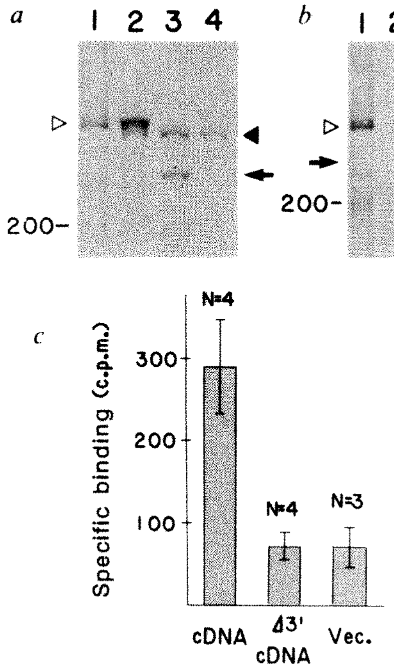


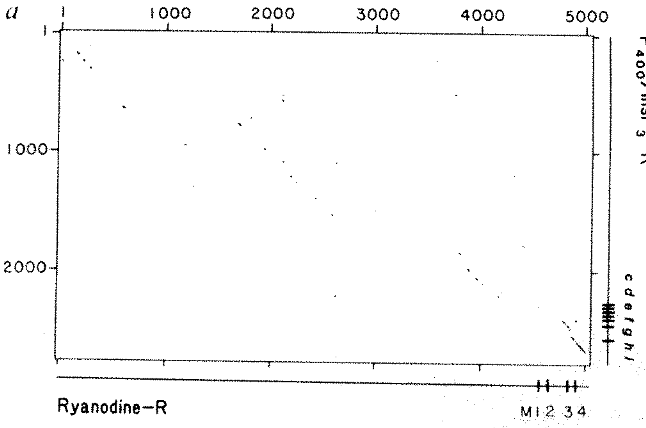
FIG. 5 Binding of $[^3H]InsP_3$ to P_{400} protein expressed in NG108-15 cells. *a*, Western blot analysis of crude membrane proteins using the anti-P_{400} monoclonal antibody 4C11. Lane 1, Microsomal proteins (5 µg) prepared from the mouse cerebellum; lane 2, crude membrane proteins (50 µg) prepared from the NG108-15 transfected with P_{400} cDNA; lane 3, 3′-deletion construct and lane 4, vector plasmid DNA. Cerebellar P_{400} protein (△); endogenous P_{400} of NG108-15 (▲); truncated P_{400} (→). The position is shown of a molecular weight marker ($\times 10^{-3}$). *b*, Western blot analysis of soluble cytosolic proteins using 4C11. Lane 1, the same as lane 1 of *a*; lanes 2 to 4, soluble cytosolic proteins (15 µg) prepared from the same cells as those in lanes 2 to 4 of *a*, respectively. *c*, Specific binding of $[^3H]InsP_3$ to crude membrane proteins prepared from NG108–15 transfected with P_{400} cDNA (cDNA), the 3′-deletion construct (Δ3′ cDNA) or vector DNA (Vec). *N* represents the number of samples tested. Results are the means ± s.e.m. of *N* samples.

METHODS. Plasmid DNAs used for the transfection assay were constructed as follows. p*β*act-CAT9 (ref. 43) was digested with *Hin*dIII and *Hpa*I to remove the chloramphenicol acetyltransferase (CAT) sequence, treated with Klenow enzyme and dNTPs, and ligated to a *Sal*I linker (GGTCGACC). The resulting plasmid pBact-S, containing a single *Sal*I site in place of the CAT gene fragment, between the *β*-actin promoter and SV40 polyadenylation sequences, was used as a vector. To generate pBact-CI, the full P_{400} cDNA (short type in Fig. 1) was ligated to *Sal*I linkers at both ends and inserted into the *Sal*I site of pBact-S. The DNA fragment from the 5′ end to an internal *Eco*RI site (6,646 nucleotide position in Fig. 1) of P_{400} cDNA was also inserted into the *Sal*I site of pBact-S. The resultant plasmid pBact-EL2 encodes a ~252K truncated polypeptide which lacks the 534 amino acids of the C-terminal portion (Fig. 2). These plasmid DNAs were introduced into NG108-15 cells by the standard calcium phosphate precipitation technique[44]. Two days after transfection, cells were collected, washed with ice-cold TBS (Tris-buffered saline) and resuspended in ice-cold 0.25M sucrose in buffer A (10 mM Tris–HCl, pH 8.0, 0.1 mM EDTA, 1 mM PMSF, 0.01 mM pepstatine A, 0.2 U ml^{-1} aprotinin). The cells were homogenized by 10 strokes in a chilled glass-Teflon Potter homogenizer and centrifuged at 3,300*g* for 3 min at 2 °C to remove nuclei. The supernatants were recentrifuged at 95,000 r.p.m. ($393,201 \times g_{max}$) in a Beckman TLA100.2 rotor for 5 min at 2 °C. The resulting pellets and supernatants were used as crude membrane proteins and soluble cytosolic proteins, respectively. Some of the protein was used for western blot analysis[6,7]. Cerebellar microsome was prepared as described[7]. The $[^3H]InsP_3$-binding assay, using rapid centrifugation[15] for membrane fractionation or PEG precipitation for soluble fractionation[15], was performed by incubating 50 µg of the proteins in 100 µl buffer A containing 10 nM $[^3H]InsP_3$ (NEN) for 10 min at 4 °C. Nonspecific binding was determined in the presence of 1 µM cold $InsP_3$. Specific binding was defined by the subtraction of nonspecific binding, ranging from 200 to 250 c.p.m., from total binding.

in NG108-15 (lane 4) is less than that in the mouse cerebellum (lane 1). This size difference allows discrimination between cerebellar P_{400} and endogenous P_{400} in NG108-15. Scatchard analysis using $[^3H]InsP_3$ revealed that the NG108-15 P_{400} differs from the cerebellar P_{400} in having two specific binding components, of high- and low-affinity, with low capacity (A.M. *et al.*, unpublished data). In the transient assay, the P_{400} cDNA-transfected cells produced a large amount of protein immunoreactive to all three of the anti-P_{400} monoclonal antibodies (Fig. 5*a*, lane 2; immunoreaction pattern with 4C11). Both the cDNA-derived and endogenous P_{400} proteins were observed in membrane fractions, but not in soluble cytosolic fractions (lane 2 in Fig. 5*a*, *b*). In the cDNA-transfected cells, expression of the cerebellar-type P_{400} protein, derived from the cDNA, is apparently coupled with the elevation of $[^3H]InsP_3$ binding activity (Fig. 5*c*). The expressed protein displays a high affinity (K_D, 21.7 nM) and specificity for $Ins(1,4,5)P_3$ with high capacity, as does P_{400} in cerebellar microsomes[11,15] (A.M. *et al.*, unpublished data). According to our preliminary data, specific $[^3H]Ins(1,4,5)P_3$ binding of the expressed protein is competed out tenfold less by $Ins(2,4,5)P_3$ than by $Ins(1,4,5)P_3$. The potency order of competition by various inositol phosphates is: $Ins(1,4,5)P_3 > Ins(2,4,5)P_3 \geq Ins(1,3,4,5)P_4 > Ins(1.4)P_2$.$Ins(1)P$ is inactive in competition at concentrations as high as 50 µM. When transfected with the 3′-deletion construct that lacks the 3′ region encoding the 534 amino-acid residues of the C-terminus, including the putative transmembrane domains (Fig. 2), NG108-15 produced a truncated protein (~252K) immunoreactive to antibodies 4C11 and 10A6, but not 18A10. Expression of the truncated protein was not accompanied by elevation of $InsP_3$-binding activity (Fig. 5*c*). Truncated protein was observed mostly in the soluble cytosolic fraction (lane 3 in Fig. 5*b*), about 1% being found in the membrane fraction (lane 3 in Fig. 5*a*), which could be due to contamination during preparation.

These results demonstrate that the P_{400} protein encoded by our cDNA is present on membranes and does possess $InsP_3$ binding activity. The results from the transfection assay with the 3′-deletion construct reveal that C-terminal hydrophobic stretches could function as a membrane anchor, because the mutant protein accumulates in the cytoplasm. The deleted region

FIG. 6 Sequence comparison between the murine $InsP_3$-binding protein P_{400} and the rabbit ryanodine receptor. *a*. Diagonal matrix comparison using a DIAGON computer program[45] modified by Dr T. Inoue. The dots correspond to mid-points of 25-residue spans, giving a double-matching probability that the mean score is ≥1.7. Solid bars indicate possible transmembrane segments. *b*. Alignment of the amino-acid sequences. Identical residues are enclosed within solid boxes and conservative substitutions within dashed boxes. Gaps (dashes) were introduced to maximize homology. The numbers of inserted residues are indicated in parentheses. c–i, Putative P_{400} membrane-spanning regions. M1–M4, putative ryanodine receptor membrane-spanning sequences.

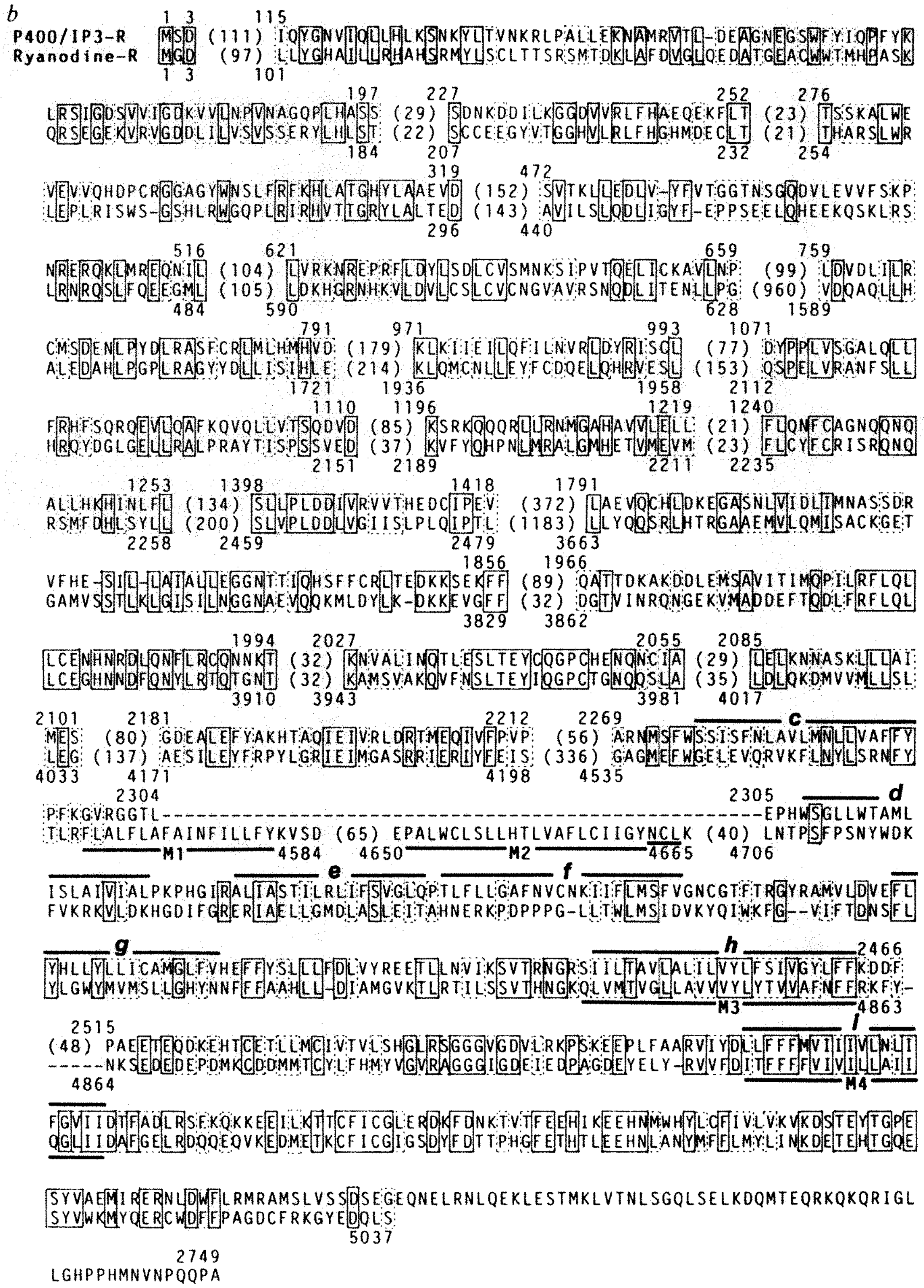


may also be responsible for $InsP_3$ binding, or may be important in forming a conformation capable of $InsP_3$ binding.

Discussion

Similar molecular features and spatial distributions suggest an identity between the P_{400} protein and the $InsP_3$ receptor/binding protein reported by Snyder *et al.*[9-12]. Recently, Nordquist *et al.*[26] isolated cDNA clones from a library of normal mouse cerebellar cDNA by differential screening with mRNA from the cerebellum of *pcd* mutant mice. We have found that one clone, PCD6, contains a 500-amino-acid sequence which has an identity with the C-terminal region of P_{400} protein, except that the leucine at residue 2,675 of P_{400} is a proline in PCD6. Distribution of PCD6 mRNA expression is also similar to that of P_{400}. These results indicate that PCD6 is a partial clone of P_{400}.

$InsP_3$ is thought to act by binding to a specific intracellular receptor, probably on a component of the ER[8] or a more discrete specialized structure, the 'calciosome'[27,28]. There are some discrepancies, however, between the subcellular localization described in our results[7] and those reported by Ross *et al.*[29]. Using polyclonal antibodies they localized the $InsP_3$ receptor to the ER, the subplasmalemmal cisternae and the nuclear membrane, but not to the plasma membrane. Our electron microscopy study using monoclonal antibody 4C11 indicates



Tyrosine phosphorylation of the fission yeast *cdc2*$^+$ protein kinase regulates entry into mitosis

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The *cdc2*$^+$ protein kinase (pp34) is found to be phosphorylated on tyrosine as well as serine and threonine residues in exponentially growing *Schizosaccharomyces pombe*. At mitosis, the level of pp34 phosphorylation on both threonine and tyrosine residues decreases. The single detectable site of tyrosine phosphorylation in pp34 has been mapped to Tyr 15, a residue within the presumptive ATP-binding domain. Substitution of this tyrosine by phenylalanine advances cells prematurely into mitosis, establishing that tyrosine phosphorylation/dephosphorylation directly regulates pp34 function.

THE *cdc2*$^+$ gene encodes a 34K phosphoprotein (that is, of relative molecular mass 34,000) with protein-serine/threonine kinase activity, termed pp34, which plays a key part in regulating the eukaryotic cell cycle (reviewed in ref. 1). In the fission yeast, *Schizosaccharomyces pombe*, *cdc2*$^+$ acts at two points in the cell cycle: first at 'start' where it is required for entry into S-phase and then again in late G2 where it regulates entry into mitosis[2]. The homologue of *cdc2*$^+$ in *Saccharomyces cerevisiae*, *CDC28*, is required at 'start'[3] and possibly later in the cell cycle for mitosis[4]. A functionally equivalent and structurally similar gene has also been identified in human cells[5,6] and other organisms[7,8]. In frog[9,10] and starfish[11], pp34 is a component of maturation promoting factor, which regulates onset of meiotic and mitotic M-phase during oocyte maturation and early embryogenesis. These observations have led to the proposal that pp34 is part of a universal control mechanism regulating onset of M-phase in all eukaryotic cells[1].

Entry into M-phase is believed to be brought about by activation of pp34 protein kinase activity, which rises to a peak at M-phase in all organisms investigated[1]. In *S. pombe*, activation of pp34 requires a 56K cyclin-like protein[12] encoded by *cdc13*$^+$ (refs 13-16) and the *cdc25*$^+$ gene product[12,17,18]. Cyclins have been found physically associated with pp34 in eukaryotic cells[19-21], and there is increasing evidence that *cdc25*$^+$-like genes function in mitotic control in other organisms[22,23]. Activation of pp34 in *S. pombe* is inhibited by the *wee1*$^+$ gene product, a putative protein-serine/threonine kinase[24]. In starfish[11], frog[25,26], and mammalian cells (refs 27, 28; C. Norbury, personal communication), protein kinase activation at M-phase is associated with pp34 dephosphorylation. Anti-phosphotyrosine antibodies have been used to demonstrate that in frog[25] and mammalian cells[28] dephosphorylation occurs on tyrosine and this has been confirmed in mammalian cells by phosphoamino-acid analysis (ref. 28; C. Norbury, personal communication). A role for tyrosine phosphorylation in regulating pp34 is of particular interest, given the importance of tyrosine-protein kinases in the control of mammalian cell growth. However, in both *S. pombe*[29] and *S. cerevisiae*[30], pp34 phosphorylation levels have been reported not to change during the cell cycle and phosphoamino-acid analyses have failed to detect phosphotyrosine in the protein[31,32]. Indeed, it is not even clear if tyrosine phosphorylation of any protein occurs in unicellular eukaryotes such as yeast, although low levels of phosphotyrosine[33] and protein-tyrosine kinase activity[34] in *S. cerevisiae* have been reported.

In this paper we establish that *S. pombe* pp34 is phosphorylated on tyrosine as well as on serine and threonine, the first demonstration of phosphotyrosine in this unicellular organism. Using highly synchronized cultures, we show that dephosphorylation of tyrosine and threonine residues occurs as *S. pombe* cells enter mitosis. We have localized the single detectable site of tyrosine phosphorylation in pp34 to Tyr 15, a residue within the proposed ATP-binding domain. Replacement of this tyrosine with phenylalanine abolishes tyrosine phosphorylation of pp34 and leads to premature advancement of cells into mitosis. This demonstrates that tyrosine phosphorylation/dephosphorylation of pp34 directly regulates entry of cells into mitosis.

Dephosphorylation during mitosis

To examine the phosphorylation state of pp34, we used an antiserum, hereafter referred to as PN24, raised against a peptide corresponding to the C-terminal seven amino acids of the *cdc2*$^+$ gene product[29]. Immunoprecipitation from ^{32}P-labelled wild-type *S. pombe* (strain 972) with PN24 revealed a major 34K band following electrophoresis and autoradiography (Fig. 1*a*, lane 1). The specificity of this reaction was demonstrated by the ability of the C-terminal peptide to block immunoprecipitation of pp34 when pre-incubated with the antiserum (Fig. 1*a*, lane 2). Also, one-dimensional peptide mapping of the immunoprecipitated 34K protein in comparison with bacterially produced pp34 using the chemical cleavage reagent, *N*-chlorosuccinimide[5] established that the immunoprecipitated protein was the product of the *cdc2*$^+$ gene (data not shown).

The relative level of pp34 phosphorylation at various points during the cell cycle was examined by immunoprecipitating pp34 from ^{32}P-labelled temperature-sensitive cdc strains arrested at their nonpermissive temperatures. Specifically, we used the *cdc25*-22 and *cdc13*-117 strains to block cells either in late G2 or in mid-mitosis, respectively. There was four times more ^{32}P in pp34 isolated from cells blocked in late G2 than there was in pp34 immunoprecipitated from cells arrested in mitosis (Fig. 1*b*, lanes 1 and 2). When isolated from exponentially growing cells, pp34 contained an intermediate level of ^{32}P (data not shown). Consistent with the lower ^{32}P content of pp34 in mitotically arrested cells, a decrease in pp34 phosphorylation was observed as cells entered mitosis when released from a late G2 block (Fig. 1*c*).

Tyrosine phosphorylation

Immunoprecipitates of pp34 from various ^{32}P-labelled cells were partially hydrolysed with acid to determine their phosphoamino-acid composition. All three phosphoamino acids were found in pp34 from exponentially growing cells (Fig. 2*a*). Phosphothreonine was most prominent, phosphotyrosine somewhat less abundant, and phosphoserine was barely detectable. The phosphoamino-acid content of pp34 from cells blocked in late G2 was slightly different in that phosphoserine was not detectable even in very long exposures and the level of phosphotyrosine was roughly equivalent to the level of phosphothreonine (Fig. 2*b*). The ratio of phosphothreonine to phosphotyrosine in pp34 was nearly 1 : 1 in cells blocked in mitosis (Fig. 2*c*) or released from a G2 block for 30 min (Fig. 2*d*), when overall phosphorylation is reduced. This indicates that dephosphorylation of both threonine and tyrosine residues occurs as cells traverse the boundary between G2 and M-phase.

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The phosphotyrosine content of pp34 as cells entered mitosis was also examined with anti-phosphotyrosine antibodies. We immunoprecipitated pp34 from unlabelled *cdc25*-22 cells arrested in late G2 at 36 °C and released for 30, 45 and 60 min by shifting to the permissive temperature of 25 °C. The amount of phosphotyrosine in pp34 decreased 4-5-fold within 30-45 min of release, whereas the level of protein did not change appreciably (Fig. 3*a, b*). This decrease in reactivity with anti-phosphotyrosine antibodies occurred with the same kinetics as the decrease in overall ^{32}P content of pp34 observed in [^{32}P]-orthophosphate labelling experiments (compare Fig. 1*c* with Fig. 3*a*).

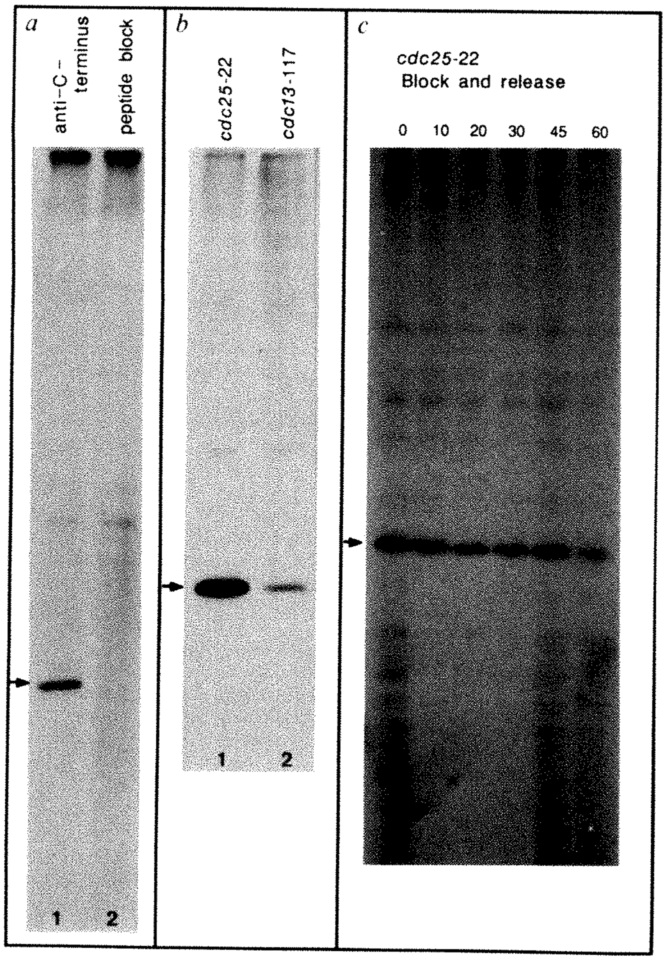


FIG. 1 Phosphorylation of pp34 during the G2-M phase transition. *a*, Wild-type *S. pombe* strain 972 was labelled with [^{32}P]orthophosphate and lysed as described[44]. The pp34 protein was immunoprecipitated with excess antibody raised against the C-terminal seven amino acids of pp34 (ref. 29) (lane 1) or with the same antibody pre-incubated with 100 ng of the C-terminal peptide (lane 2). *b*, Immunoprecipitates of pp34 from equivalent amounts of ^{32}P-labelled *cdc25*-22 (lane 1) or *cdc13*-117 (lane 2) cells arrested at 36 °C. *c*, Immunoprecipitates of pp34 from equal numbers of *cdc25*-22 cells labelled with [^{32}P]orthophosphate for 4.5 h at 36 °C and shifted to 25 °C for 0, 10, 20, 30, 45 and 60 min. Arrows indicate the position of pp34.

METHODS. *S. pombe cells* (5 or 10 ml) at 2-4 $\times 10^6$ cells per ml were labelled with 1-3 mCi [^{32}P]orthophosphate in reduced phosphate minimal medium as described earlier[29,44]. Wild-type strain 972 was labelled at 30 °C for 4 h and *cdc25*-22 and *cdc13*-117 strains at 36 °C for 4 or 4.5 h. Exponentially growing unlabelled wild-type cells (10^8) were added and lysis was performed as reported[29] with modifications[44]. The pp34 protein was visualized by autoradiography after separation on SDS-17.5% polyacrylamide gels. Exposure was for 3-16 h at −70 °C with intensifying screens. Gel bands were quantitated by scintillation counting.

Tyrosine phosphorylation site

As a first step towards identifying the site(s) of tyrosine phosphorylation, we examined the phosphopeptide composition of pp34. The patterns of pp34 tryptic phosphopeptides were qualitatively identical when derived from exponentially growing wild-type *S. pombe* cells (Fig. 4*a*) or from cells arrested in late G2 (Fig. 4*b*). The pp34 protein contained one major phosphopeptide, termed phosphopeptide 1, and a cluster of minor peptides, collectively termed phosphopeptide 2 (Fig. 4*a, b*). Phosphopeptide 1 contained only phosphotyrosine (Fig. 4*c*) and was the only peptide to contain detectable levels of phosphotyrosine. Phosphopeptide 2 contained phosphothreonine (Fig. 4*d*). The low yield of the phosphothreonine-containing peptide relative to the phosphotyrosine-containing peptide is probably due to the relative insolubility of phosphopeptide 2 in our buffer system, a characteristic of large hydrophobic peptides. We were unable to detect a phosphoserine-containing tryptic phosphopeptide, presumably because of insufficient ^{32}P-labelled sample.

We were able to localize the tryptic peptide-containing phosphotyrosine to the N-terminal 3-4K fragment of the protein based on the following observations. First, *N*-chlorosuccinimide cleaves pp34 to generate a characteristic pattern of partial cleavage products ranging from ~18-32K (ref. 5). All of these fragments contain the N-terminus of the protein (ref. 5; unpublished observations). Because we found that all of the ^{32}P-labelled pp34 *N*-chlorosuccinimide fragments contained phosphotyrosine (data not shown), the tyrosine phosphorylation site had to lie within the 18K N-terminal fragment of the protein. Second, the largest fragment (~28K) derived from pp34 by partial *S. aureus* V8 protease digestion, fragment A (Fig. 4*e*), lacked phosphotyrosine (Fig. 4*g*). Because fragment A is generated by removal of a section of ~4K from the N-terminus of the protein (data not shown), the site of tyrosine phosphorylation was most probably in this ~4K N-terminal piece of pp34.

There are three tyrosine residues in this region of the protein which lie in two tryptic peptides[35]. One tryptic peptide (amino acids 1-6) contains Tyr 4 and the other (amino acids 10-20) contains both Tyr 15 and Tyr 19. The tryptic peptide containing Tyr 4 was unlikely to be phosphopeptide 1 for two reasons. First, its theoretical mobility in our two-dimensional peptide mapping system did not correspond to the migration of phosphopeptide 1. Second, phosphopeptide 1 comigrated in our two-dimensional separations with a phosphotyrosine-containing tryptic peptide derived from murine pp34, strongly suggesting that a peptide with the identical sequence is phosphorylated in murine pp34 (C. Norbury, K.L.G. and P.N., unpublished observations). Comparison of the murine[7] and *S. pombe* pp34 sequences[35] revealed that the only tryptic peptide in the relevant N-terminal region of pp34 present in both murine and *S. pombe* pp34 was the peptide containing Tyr 15 and Tyr 19.

To establish that phosphopeptide 1 contained amino acids 10-20, we synthesized this ^{32}P-labelled tryptic peptide *in vitro* and compared its migration in two-dimensional separations with that of phosphopeptide 1. To generate this ^{32}P-labelled tryptic peptide *in vitro*, a peptide corresponding to amino acids 7-26 of pp34 was synthesized (Fig. 5*a*), phosphorylated by immunoprecipitates of pp60$^{v\text{-}src}$ (Fig. 5*b*) on tyrosine (data not shown), and cleaved with trypsin. The resultant synthetic tryptic phosphopeptide (Fig. 5*c*, panel *A*) comigrated with phosphopeptide 1 (Fig. 5*c*, panel *C*). These data indicated that phosphopeptide 1 was a phosphotyrosine-containing peptide corresponding to amino acids 10-20.

From the migration of phosphopeptide 1 at different pHs, it appeared to contain only one phospate moiety (data not shown) indicating that Tyr 15 and Tyr 19 were not phosphorylated in the same peptide molecule. To rule out the possibility that phosphopeptide 1 consisted of a mixture of two phosphopeptides, one phosphorylated at Tyr 15 and the other at Tyr 19, phosphopeptide 1 was cleaved with thermolysin. As shown in Fig. 5*a*, there are two potential thermolytic cleavage sites between Tyr 15 and Tyr 19. If both tyrosine residues were phosphorylated, cleavage with thermolysin would generate at least two distinct phosphopeptides. However, as phosphopeptide 1 yielded only one phosphopeptide on thermolytic cleavage (Fig. 5*c*, panels *D* and *E*), then only one tyrosine residue could be the site of phosphorylation *in vivo*.

Tyrosine substitutions

The codons for Tyr 15 and Tyr 19 were changed independently by site-directed mutagenesis to encode the structurally similar amino acid phenylalanine. The wild-type and mutant proteins were expressed from multicopy plasmids in a strain in which the *cdc2* gene had been deleted. These cells were labelled with [^{32}P]orthophosphate and the phosphoamino-acid composition of the *cdc2* proteins was examined. Although the *cdc2*$^+$ and *cdc2.Phe19* proteins contained all three phosphoamino acids (Fig. 6*a*, panels *A* and *C*), phosphotyrosine was absent in the *cdc2.Phe15* protein (Fig. 6*a*, panel *B*). This result confirmed that Tyr 15 was the site of phosphorylation.

The phenotypes of cells deleted for *cdc2*$^+$ and expressing plasmid-borne *cdc2*$^+$ or *cdc2.Phe19* were indistinguishable from that of wild-type cells (Fig. 6*b*, panels *A* and *B*). In contrast, cells expressing *cdc2.Phe15* grew very slowly and had the general appearance of '*wee*' mutants[36,37], septating at an average length of 7 μm rather than at the wild-type size of 14 μm (Fig. 6*b*, panels *C*-*F*). An abnormally high percentage of the cells contained septa (some very wide) and a large proportion of the cells were either anucleate, binucleate, had nuclei that were cut by septa, or had fragmented nuclei (Fig. 6*b*, panels *C*-*F*). These pleiotropic and deleterious effects of the *cdc2.Phe15* mutant are not observed in other single *wee* mutants such as *wee1*-50, *cdc2*-1w, or *cdc2*-3w (refs 36, 37). However, they are similar to the effects generated by the double mutant, *wee1*-50 *cdc2*-3w (ref. 24). This strain undergoes mitotic catastrophe at the *wee1*-50 restrictive temperature; the cells enter mitosis so early that

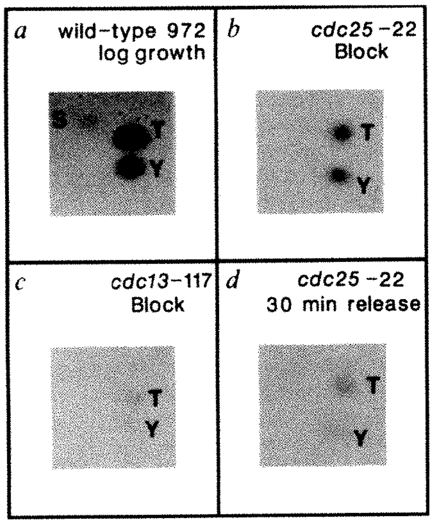


FIG. 2 Phosphoamino-acid compositions of pp34: *a*, pp34 from exponentially growing wild-type cells; *b*, pp34 from *cdc25*-22 cells labelled at 36 °C for 4.5 h; *c*, pp34 from *cdc25*-22 cells labelled at 36 °C and shifted to 25 °C for 30 min; *d*, pp34 from *cdc13*-117 cells labelled at 36 °C. Key: S, phosphoserine; T, phosphothreonine; Y, phosphotyrosine.

METHODS. ^{32}P-labelled pp34 immunoprecipitates were obtained as described in the legend to Fig. 1, subjected to partial acid hydrolysis, and the phosphoamino acids were resolved by two-dimensional thin-layer electrophoresis as described[45,46]. The sample of wild-type pp34 was derived from twice the number of cells labelled with more [^{32}P]orthophosphate. Samples containing 50-350 c.p.m. were loaded onto thin-layer cellulose and exposed for 6 days at −70 °C with intensifying screens. Thin-layer electrophoresis was performed on a CBS Scientific apparatus.

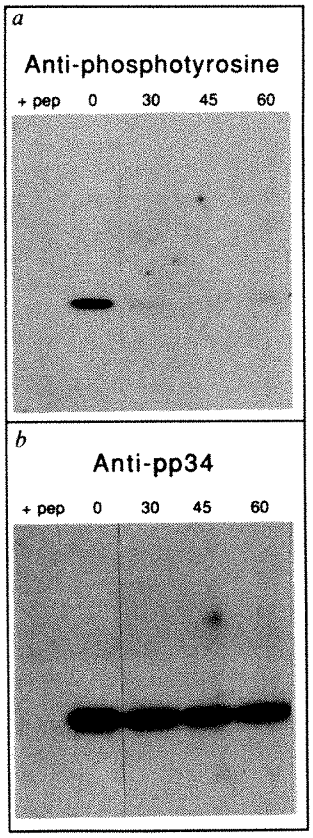
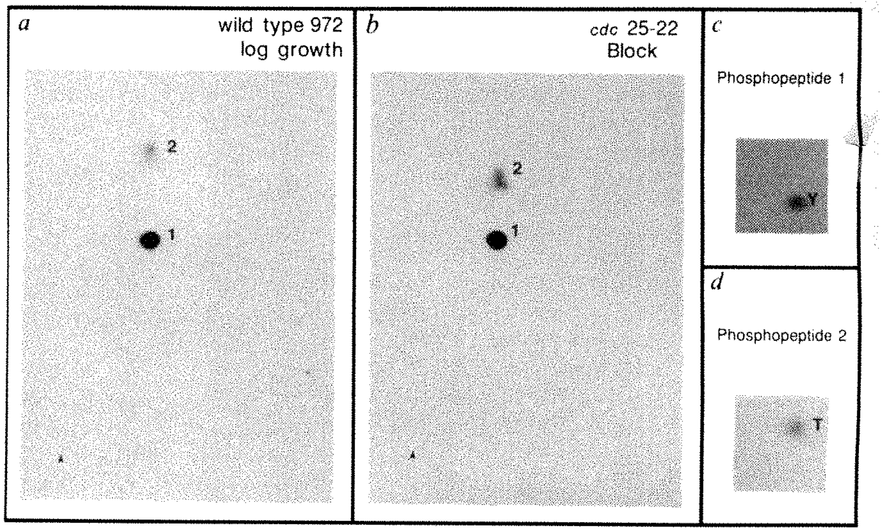


FIG. 3 Western blots of pp34 immunoprecipitates. Exponentially growing *cdc25*-22 cells at 3×10^6 cells per ml were arrested at 36 °C for 4.5 h. At each time interval (0, 30, 45 and 60 min) after shift to 25 °C, 10^8 cells were lysed and subjected to immunoprecipitation with the anti-C-terminal peptide serum or at time 0 with the anti-C-terminal peptide serum pre-incubated with excess peptide as described in the legend to Fig. 1. Each immunoprecipitate was divided in half, and each half was resolved separately on SDS-17.5% polyacrylamide gels, blotted onto Immobilon-P, and probed with either anti-phosphotyrosine antibodies (*a*) or anti-pp34 antibodies (*b*), followed by ^{125}I-labelled protein A. The main band corresponds to pp34.

METHODS. The 3.4-kilobase *Pst*I genomic fragment of *cdc2*$^+$ was cloned into the vector pTZ19R (Pharmacia). The four introns in *cdc2*$^+$ were removed and a *Nde*I site was created at the initiating methionine by site-directed mutagenesis as described[5,47]. A *Bam*HI site was introduced 36 base pairs downstream of the stop codon by changing the sequence CTATCC to GGATCC by site-directed mutagenesis. All site-directed mutagenesis was carried out with an Amersham *in vitro* mutagenesis kit according to the manufacturer's instructions. The *Nde*I-*Bam*HI *cdc2*$^+$ fragment was subcloned into the bacterial expression vector pRK171 and expressed in the bacterial strain BL21(DE3) as reported previously[5,31]. After lysis of the bacteria with a French press, *cdc2* protein was purified from insoluble inclusion bodies by solubilization in gel sample buffer and electroelution from SDS-polyacylamide gels. Polyclonal rabbit serum was raised in rabbits by injection of 500 μg of pp34 near the popliteal lymph node first in complete Freund's adjuvant and thereafter every four weeks with the same amount of protein mixed with incomplete Freund's adjuvant. One antiserum (4711) was used at a 1:500 dilution for western blotting. Western blotting with anti-pp34 antibodies and anti-phosphotyrosine antibodies (from M. Kamps and B. Sefton) was performed as described[48], except that 10 μCi of ^{125}I-labelled protein A at 2-10 μCi per μg protein A was used to detect rabbit immunoglobulins. Exposures were for 10 days for *a* and 3 days for *b* at −70 °C with intensifying screens.



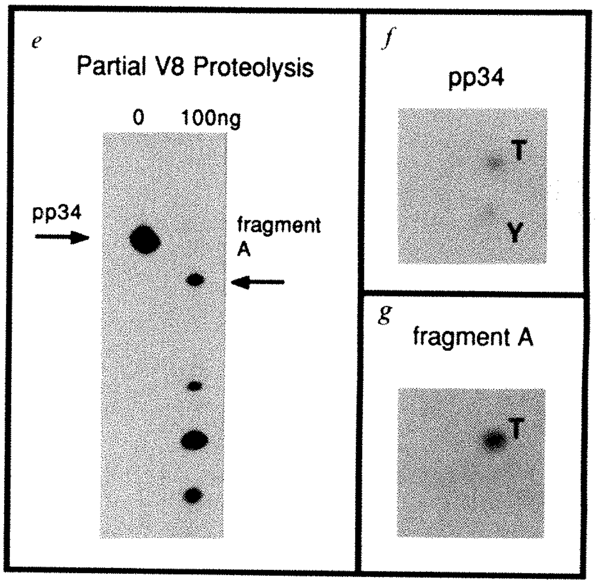


FIG. 4 Tryptic peptide and partial *S. aureus* V8 peptide analysis of pp34. *a*, Tryptic phosphopeptides of pp34 from ^{32}P-labelled exponentially growing 972 cells. *b*, Tryptic phosphopeptides of pp34 from ^{32}P-labelled *cdc25-22* cells arrested at 36 °C. Electrophoresis was at pH 4.72 in the horizontal dimension, with the anode on the left. The origins are indicated with arrowheads. *c*, Phosphoamino-acid analysis of phosphopeptide 1. *d*, Phosphoamino-acid analysis of phosphopeptide 2. *e*, One-dimensional peptide map of pp34 from *cdc25-22* cells arrested at 36 °C treated with 0 or 100 ng *S. aureus* V8 protease. *f*, Phosphoamino-acid analysis of undigested pp34. *g*, Phosphoamino-acid analysis of pp34 V8 fragment A.

METHODS. ^{32}P-labelled pp34 immunoprecipitates were subjected to tryptic digestion, and the tryptic phosphopeptides were separated in two dimensions as detailed previously[46,49]. Phosphoamino-acid analysis was as described in the legend to Fig. 2. One-dimensional peptide maps of pp34 were prepared on SDS-20% polyacrylamide gels with 0 or 100 ng of *S. aureus* V8 protease (Boehringer) as described[49]. Fragment A yielded the same number of *N*-chlorosuccinimide products as intact pp34 and reacted with PN24, but not an antipeptide serum directed against the N-terminal seven amino acids of the protein[29] (data not shown). This demonstrated that fragment A was generated by cleavage of an N-terminal peptide from the protein. Exposure times were 4–6 days at −70 °C with intensifying screens. Cerenkov c.p.m. loaded onto thin-layer plates ranged from 50–350 c.p.m.

cell death frequently results. Like this double mutant, *cdc2.Phe15* appears to be independent of some inhibitory signal(s) which normally delays entry into mitosis until all other necessary steps in the cell cycle have been completed. The very slow growth of the *cdc2.Phe15* strain can be accounted for by the large percentage of inviable cells which are produced in each generation from lethal premature entry into mitosis. When *cdc2.Phe15* was expressed in *cdc2*$^+$ strains, the same phenotype was observed (Fig. 6*b*, panels *G* and *H*), demonstrating that the mutant is dominant over wild-type *cdc2*$^+$ when present on a multicopy plasmid. Also, *cdc2.Phe15* is able to rescue the temperature-sensitive mutant *cdc2*-33, providing further evidence that it encodes a functional *cdc2* protein. In addition, like the *wee* mutant *cdc2*-3w, it is able to rescue the conditionally lethal *cdc25-22* strain.

Discussion

We have shown in this paper that *S. pombe* pp34 is multiply phosphorylated in exponentially growing cells. It is maximally phosphorylated in late G2 and as cells enter mitosis, the level of pp34 phosphorylation decreases. The protein kinase activity of pp34 in *S. pombe* was shown in earlier reports[12,17] to increase from a low level in late G2 (at a *cdc25*-22 block point) to its maximum level in mitosis (during a *cdc25*-22 release) with the same kinetics as described here for dephosphorylation. A decrease in pp34 phosphorylation as cells enter mitosis has been observed previously in starfish[11], frog[25,26], and mammalian[27,28] cells. Because dephosphorylation in these higher eukaryotes is coincident with an increase in pp34 protein kinase activity towards histone H1, it has been proposed that dephosphorylation might regulate the activation of pp34 (refs 11, 25, 26, 28). The fact that pp34 enzymatic activity is inversely correlated with its level of phosphorylation during the G2-M transition in *S. pombe* and in several higher eukaryotes indicates that this potential regulatory mechanism has been highly conserved throughout eukaryotic evolution.

A previous investigation in our laboratory did not detect any changes in pp34 phosphorylation levels as *S. pombe* cells moved through the cell cycle[29]. The most likely explanation for the apparent discrepancy between our two reports lies in the methods used for obtaining synchronous cell cultures. In the present study, we used the temperature-sensitive mutations *cdc25*-22 and *cdc13*-117, which arrest either in late G2 or mitosis, respectively. On shift from the restrictive to the permissive temperature, *cdc25*-22 cells enter mitosis in a highly synchronous fashion. In the earlier study, cells were fractionated by size using an elutriation rotor[29]. Although this method causes the least physiological disturbance to the cells, it does not achieve such good cell-cycle synchrony. If pp34 dephosphorylation in *S. pombe* is quite transient and cells arrived at a point in mitosis when dephosphorylation occurs somewhat asynchronously in our experiments, we would only expect to see a small overall change. Because we observed only a fourfold dephosphorylation of pp34 in cultures synchronized by a *cdc25*-22 block and release, it is likely that dephosphorylation would have been extremely difficult to detect in cultures synchronized by elutriation. A small change in overall pp34 phosphorylation might also indicate that only a subpopulation of pp34 molecules in *S. pombe* is subject to dephosphorylation during the G2-M transition. Like the previous study on pp34 phosphorylation in *S. pombe*[29], phosphorylation changes were not detected in the *S. cerevisiae* homologue of pp34 during the cell cycle[30]. More recently, however, a transient phosphorylation of the *CDC28* gene product on tyrosine residues has been observed (S. Reed, personal communication).

We have found both by phosphoamino-acid analysis and by immunoblotting with anti-phosphotyrosine antibodies that pp34 contains phosphotyrosine. The phosphotyrosine content of pp34 is maximal in late G2 and decreases with the same kinetics as total ^{32}P content of the protein when cells enter mitosis. Phosphotyrosine was not detected in a previous study of pp34 phosphorylation, although phosphothreonine and phosphoserine were detected in roughly the same proportion that we have observed[31]. We do not know the reason for this discrepancy but suspect that it is due to the very different labelling conditions and cell densities used.

The *cdc2*$^+$ protein kinase is the first yeast protein known to contain phosphotyrosine. There has been a report of an unknown protein reacting with anti-phosphotyrosine antibodies in yeast lysates but this has not been substantiated by phosphoamino-acid analysis[38]. Because anti-phosphotyrosine antibodies have been reported to react in some circumstances with phosphohistidine, phosphothreonine and phosphoserine residues within proteins (ref. 39; R. Lindberg and T. Hunter, personal communication), reports relying solely on reactivity with anti-phosphotyrosine antibodies have been regarded with some scepticism. Very small amounts of phosphotyrosine have been obtained from partial acid hydrolysates of total cell protein[33]. However, other modifications of tyrosine such as adenylylation can yield phosphotyrosine upon partial acid hydrolysis[40]. Thus, whether the tiny amounts of phosphotyrosine detected in acid hydrolysates are actually due to modification of tyrosine residues by enzymatic phosphorylation remains an open issue. Tyrosine-protein kinase activity in yeast, on the other hand, has been demonstrated more definitively. A very low level of activity that phosphorylates a copolymer of glutamic acid and tyrosine on tyrosine has been identified in crude lysates of *S. cerevisiae*[34]. In multicellular eukaryotes, tyrosine phosphorylation has been more readily detected and is intrinsic to more than 30 proteins[41]. Of the diverse array of protein-tyrosine kinases known, most appear to be associated with the plasma membrane, either as growth factor receptors or as putative transducers of extracellular stimuli[41]. Because such a highly elaborate system for responding to growth factors and other intercellular signals is unlikely to be required in a unicellular organism, it is probable that many fewer protein-tyrosine kinases exist in yeast. However,

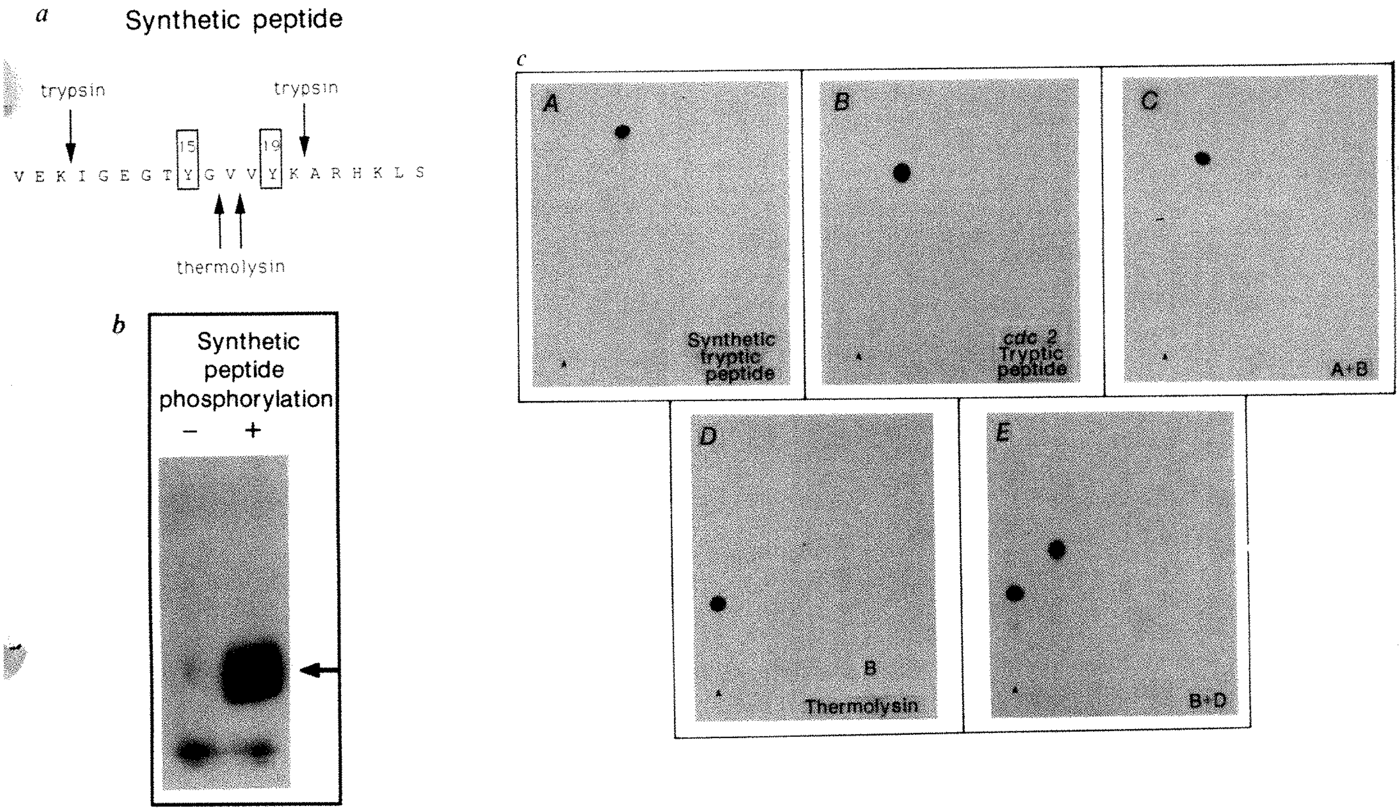

a
Synthetic peptide
trypsin
trypsin
15
19
V E K I G E G T Y G V V Y K A R H K L S
thermolysin
b
Synthetic peptide phosphorylation
–
+
c
A
Synthetic tryptic peptide
B
cdc 2 Tryptic peptide
C
A+B
D
B
Thermolysin
E
B+D

FIG. 5 Localization of the tyrosine phosphorylation site in pp34. *a*, Sequence of the synthetic peptide corresponding to amino acids 7–26 of pp34. Arrows indicate cleavage sites for trypsin and thermolysin. The two tyrosines are boxed. *b*, Phosphorylation of the synthetic peptide by pp60$^{v\text{-}src}$ in the presence of [γ-^{32}P]ATP. The plus and minus signs refer to reactions which did or did not contain the peptide, respectively. The arrow indicates the position of phosphorylated peptide. *c*, The synthetic peptide phosphorylated by pp60$^{v\text{-}src}$ was cleaved with trypsin and resolved in two dimensions (panel *A*). The tryptic phosphopeptide containing phosphotyrosine shown in Fig. 4, *a* and *b*, was re-run separately (panel *B*). Equal Cerenkov c.p.m. of each tryptic phosphopeptide shown separately in panels *A* and *B* were mixed in panel *C*. The tryptic phosphopeptide shown in *B* was cleaved with thermolysin and resolved in two dimensions (panel *D*). Equal Cerenkov c.p.m. of each phosphopeptide run separately in panels *B* and *D* were mixed in panel *E*. Cerenkov c.p.m. loaded onto cellulose plates ranged from 60–100 c.p.m. Exposure times were 5 days at −70 °C with intensifying screens. Electrophoresis was at pH 1.9 in the horizontal dimension with the anode on the left.

METHODS. A peptide corresponding to amino acids 7–26 of pp34 was synthesized using a Applied Biosystems 430A synthesizer and purified by reverse-phase HPLC. Amino-acid analysis confirmed the authenticity of the sequence. Immunoprecipitation of pp60$^{v\text{-}src}$ was from LA29-transformed rat-1 cells with monoclonal antibody 327 (ref. 50; provided by T. Hunter) as described previously[49]. Immunoprecipitates of pp60$^{v\text{-}src}$ were resuspended in 30 μl 20 mM Tris-HCl, pH 7.5, 10 mM $MnCl_2$ and 3 μl 100 mM Tris-HCl, pH 7.5, with or without 3 μg of the synthetic peptide. Reactions were initiated by the addition of 30 μCi [γ-^{32}P]ATP and allowed to proceed at room temperature for 15 min. Immune complexes were pelleted by brief centrifugation and 10 μl of the supernatants were added to 10 μl of 2 × sample buffer. After heating to 100 °C for 2 min, this 20-μl portion was resolved on an SDS-25% polyacrylamide gel and the phosphorylated peptide was visualized by autoradiography. The remainder of the synthetic peptide phosphorylated by pp60$^{v\text{-}src}$ was purified by cellulose thin-layer electrophoresis at pH 1.9. It was eluted from the cellulose with pH 1.9 buffer, lyophilized, resuspended in 20 μl 50 mM ammonium bicarbonate, pH 8.0, and digested with 2 μg trypsin for 2 h at 37 °C. Phosphopeptide 1 (Fig. 4*a*, *b*) was eluted from cellulose plates and digested with thermolysin as described above, except that cleavage was performed at 55 °C. All other manipulations of phosphopeptides, including two-dimensional separation, were carried out as described previously[46,49].

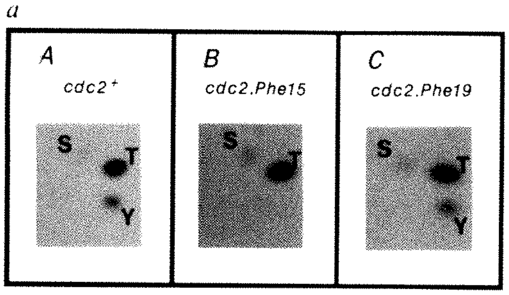
the protein-tyrosine kinases that are present in yeast might have fundamental roles in the assessment of growth and/or nutritional status and relate that information to progress through the mitotic cell cycle.

There is a single detectable site of tyrosine phosphorylation in fission yeast pp34 and we have identified it as Tyr 15. This residue lies directly within a part of the postulated nucleotide binding domain of pp34, the GXGXXG motif[42]. The location of Tyr 15 leads us to suggest that the function of tyrosine phosphorylation in pp34 is to prevent ATP binding either sterically and/or by electrostatic repulsion, thereby inactivating the protein. This hypothesis is consistent with the observation that dephosphorylation of pp34 correlates with increased protein kinase activity. Modulation of nucleotide-binding ability by phosphorylation would represent a novel mechanism of protein kinase regulation. A comparison of protein kinase sequences[42] reveals that *cdc2*$^+$ and its functional homologues are not unique in containing a tyrosine at this position. It is possible, therefore, that other protein-serine/threonine and protein-tyrosine kinases might be regulated by tyrosine phosphorylation within their ATP-binding domains.

The potential role of tyrosine phosphorylation in the regulation of pp34 was addressed by changing the codon specifying Tyr 15 to that encoding phenylalanine and then examining the effect of this mutation in *S. pombe*. Based on the observation that *cdc2.Phe15* was able to rescue a temperature-sensitive allele of *cdc2*, as well as a strain in which the *cdc2*$^+$ gene had been disrupted (although the growth rate of the rescued strains was low), we conclude that the mutant *cdc2.Phe15* protein retains enzymatic activity. Cells expressing *cdc2.Phe15* either with or without *cdc2*$^+$ entered mitosis at a small cell size and exhibited a wide range of abnormal phenotypes suggestive of mitotic catastrophe. Many were anucleate, binucleate, had septation defects, or had fragmented nuclei. Cell division in this strain resulted in many inviable progeny, accounting for its slow doubling time. The ability of this strain to grow at all indicates that this single mutant is not quite as detrimental as the double mutant *wee1*-50 *cdc2*-3w. As mentioned earlier, the combination of *wee1*-50 and *cdc2*-3w alleles results in mitotic catastrophe preventing formation of colonies at the *wee1*-50 nonpermissive temperature[24]. Thus, although *cdc2.Phe15* appears to bypass much of the normal inhibition acting to time entry into mitosis properly, this mutant does not appear to be de-regulated completely.

The *cdc2.Phe15* mutant protein is still phosphorylated on threonine and serine residues. We have shown in this report that phosphothreonine as well as phosphotyrosine decreases as cells enter mitosis, suggesting that phosphothreonine might also be involved in the regulation of pp34 activity. It will be interesting to identify the sites of threonine and serine phosphorylation and assess the role of these phosphorylation events in the regulation of pp34.

Because pp34 is regulated by tyrosine phosphorylation and dephosphorylation, presumably at least one protein-tyrosine kinase and phosphatase contribute to regulation of the *S. pombe* mitotic cell cycle. The *cdc25* gene function, which normally is required for activation of pp34, is bypassed by *cdc2.Phe15* because *cdc2.Phe15* can rescue a temperature-sensitive mutation of this gene. Although the function of *cdc25* is unknown and its amino-acid sequence does not reveal similarities to the sequence of any published protein phosphatase or protein kinase inhibitor[18], these findings would be consistent with a role for *cdc25* in the activation of a protein-tyrosine phosphatase or the inhibition of a protein-tyrosine kinase.



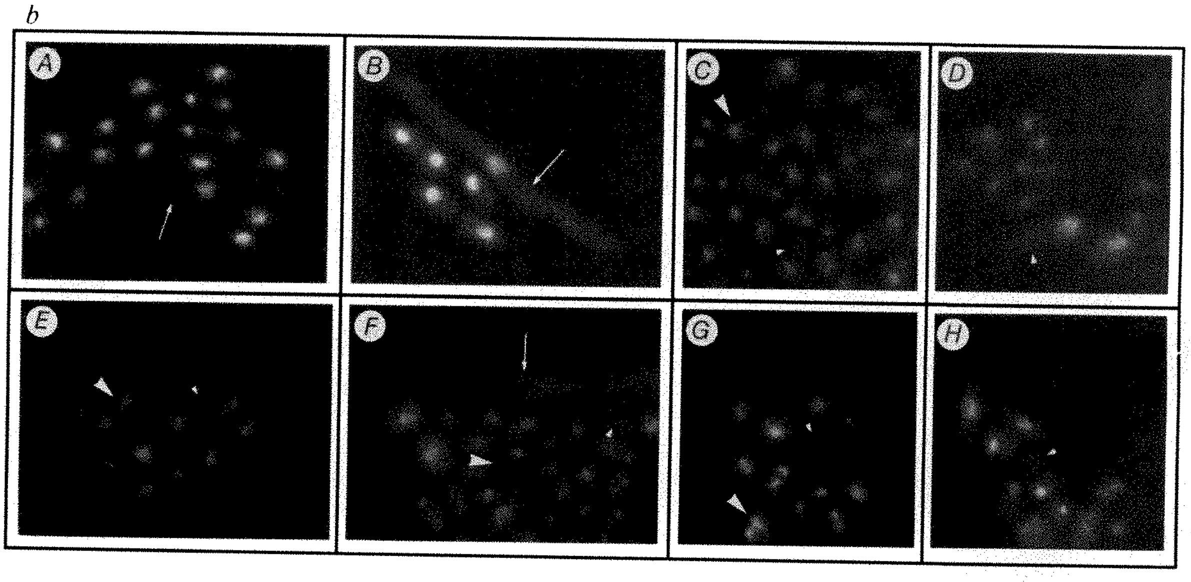


FIG. 6 Phenotype of *cdc2* mutants. *a*, Phosphoamino-acid analyses of pp34 proteins from *S. pombe* cells with a deleted *cdc2*$^+$ gene complemented by plasmid-borne *cdc2*$^+$, *cdc2.Phe15*, or *cdc2.Phe19* labelled with [^{32}P]-orthophosphate. *b*, DAPI staining. *S. pombe* cells with a deleted *cdc2*$^+$ gene complemented by plasmid-borne (*A*) *cdc2*$^+$, (*B*) *cdc2.Phe19*, or (*C, D, E* and *F*) *cdc2.Phe15*. *S. pombe leu1*-32 cells complemented by a plasmid containing *LEU2* and *cdc2.Phe15* (*G* and *H*). Long arrows indicate cells which have lost *cdc2*-containing plasmids and are *cdc*$^-$. Arrowheads point to cells undergoing abnormal septation and anucleate cells.

METHODS. Mutagenesis of Tyr 15 and Tyr 19 in *cdc2*$^+$ was carried out as described in the legend to Fig. 3 with 20-base-long mutagenic oligonucleotides. The mutant genes were transformed into the diploid strain, h^+/h^{90}, *leu1*-32/*leu1*-32, *ura 4*-D18/*ura 4*-D18, *cdc2*$^+$/*cdc2::ura4*$^+$, *ade6*-704/*ade6*-704, on a multicopy plasmid containing *LEU2* (ref. 44). *Leu*$^+$ transformants were allowed to sporulate and unsporulated cells were digested with helicase as described[44]. Spores were plated onto minimal plates lacking leucine and uracil. Colonies obtained from this selection were transferred to complete YEA medium[44]. The same vector was also used to transform *leu1*-32 cells with *cdc2.Phe15*, and *leu*$^+$ transformants were selected. ^{32}P-labelling, immunoprecipitation and phosphoamino-acid analysis were performed as described in the legends to Figs 1 and 2. DAPI staining was performed as reported elsewhere[44].

The tyrosine residue that is phosphorylated in pp34 is conserved among all known pp34 homologues from yeast to humans[6-8,32,35] and it is now known that pp34 contains phosphotyrosine in human[43], mouse[28], frog[25], *S. pombe* (this report) and *S. cerevisiae* (S. Reed, personal communication) cells. It is possible, therefore, that the tyrosine residue phosphorylated in *S. pombe* pp34 is also phosphorylated in pp34 from other species and that control of pp34 activity by dephosphorylation of this residue might be conserved throughout evolution. Purified mammalian pp34 can be phosphorylated on tyrosine by $pp60^{v\text{-}src}$ *in vitro*[43], as can a synthetic peptide corresponding to amino acids 7-26 of *S. pombe* pp34. However, it should be kept in mind that $pp60^{v\text{-}src}$ is capable of phosphorylating nonphysiologically relevant protein substrates *in vitro*[41] and that there is no evidence as yet that $pp60^{c\text{-}src}$ directly phosphorylates pp34 *in vivo* in any system. The identification and characterization of the putative protein-tyrosine kinase and phosphotyrosine phosphatase responsible for regulating pp34 phosphotyrosine levels in *S. pombe* should enhance our understanding of cell-cycle control in all eukaryotes. □

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1. Nurse, P. *Nature* (in the press).
2. Nurse, P. & Bissett, Y. *Nature* **292,** 558–560 (1981).
3. Reed, S. *Genetics* **95,** 561–577 (1980).
4. Piggott, J. R., Rai, R. & Carter, B. L. A. *Nature* **298,** 391–393 (1982).
5. Draetta, G., Brizuela, L., Potashkin, J. & Beach, D. *Cell* **50,** 319–325 (1987).
6. Lee, M. G. & Nurse, P. *Nature* **327,** 31–35 (1987).
7. Cisek, L. J. & Corden, J. L. *Nature* **339,** 679–684 (1989).
8. Krek, W. & Nigg, E. A. *EMBO J.* **8,** 3071–3078 (1989).
9. Dunphy, W. G., Brizuela, L., Beach, D. & Newport, J. *Cell* **54,** 423–431 (1988).
10. Gautier, J., Norbury, C., Lohka, M., Nurse, P. & Maller, J. *Cell* **54,** 433–439 (1988).
11. Labbe, J. C. *et al. Cell* **57,** 253–263 (1989).
12. Moreno, S., Hayles, J. & Nurse, P. *Cell* **58,** 361–372 (1989).
13. Booher, R. N. & Beach, D. *EMBO J.* **7,** 2321–2327 (1988).
14. Goebl, M. & Byers, B. *Cell* **54,** 739–740 (1988).
15. Hagan, I., Hayles, J. & Nurse, P. *J. Cell Sci.* **91,** 587–595 (1988).
16. Solomon, M., Booher, R., Kirschner, M. & Beach, D. *Cell* **54,** 738–739 (1988).
17. Booher, R. N., Alfa, C. E., Hyams, J. S. & Beach, D. H. *Cell* **58,** 485–497 (1989).
18. Russell, P. & Nurse, P. *Cell* **45,** 145–153 (1986).
19. Draetta, G. *et al. Cell* **56,** 829–838 (1989).
20. Meijer, L. *et al. EMBO J.* **8,** 2275–2282 (1989).
21. Pines, J. & Hunter, T. *Cell* **58,** 833–846 (1989).
22. Edgar, B. A. & O'Farrell, P. H. *Cell* **57,** 177–187 (1989).
23. Russell, P., Moreno, S. & Reed, S. I. *Cell* **57,** 295–303 (1989).
24. Russell, P. & Nurse, P. *Cell* **49,** 559–567 (1987).
25. Dunphy, W. G. & Newport, J. W. *Cell* **58,** 181–191 (1989).
26. Gautier, J., Matsukawa, T., Nurse, P. & Maller, J. *Nature* **339,** 626–629 (1989).
27. Draetta, G. & Beach, D. *Cell* **54,** 17–26 (1988).
28. Morla, A. O., Draetta, G., Beach, D. & Wang, J. Y. J. *Cell* **58,** 193–203 (1989).
29. Simanis, V. & Nurse, P. *Cell* **45,** 261–268 (1986).
30. Hadwiger, J. A. & Reed, S. I. *Molec. cell. Biol.* **8,** 2976–2979 (1988).
31. Potashkin, J. A. & Beach, D. *Curr. Genet.* **14,** 235–240 (1988).
32. Reed, S. I., Hadwiger, J. A. & Lorincz, A. T. *Proc. natn. Acad. Sci. U.S.A.* **82,** 4055–4059 (1985).
33. Castellanos, R. M. P. & Mazon, M. J. *J. biol. Chem.* **260,** 8240–8242 (1985).
34. Schieven, G., Thorner, J. & Martin, G. S. *Science* **231,** 390–393 (1986).
35. Hindley, J. & Phear, G. A. *Gene* **31,** 129–134 (1984).
36. Fantes, P. A. *J. Bact.* **146,** 746–754 (1981).
37. Nurse, P. & Thuriaux, P. *Genetics* **96,** 627–637 (1980).
38. Grandori, R., Vai, M., Di Renzo, M. F., Alberghina, L. & Popolo, L. *Biochem. biophys. Res. Commun.* **160,** 887–896 (1989).
39. Frackelton, A. R. Jr, Ross, A. H. & Eisen, H. N. *Molec. cell. Biol.* **3,** 1343–1352 (1983).
40. Shapiro, B. M. & Stadtman, E. R. *J. biol. Chem.* **243,** 3769–3771 (1968).
41. Hunter, T. & Cooper, J. A. *A. Rev. Biochem.* **54,** 897–930 (1985).
42. Hanks, S. K., Quinn, A. M. & Hunter, T. *Science* **241,** 42–52 (1988).
43. Draetta, G. *et al. Nature* **336,** 738–744 (1988).
44. Moreno, S., Nurse, P. & Klar, A. *Meth. Enzym.* (in the press).
45. Cooper, J. A., Sefton, B. M. & Hunter, T. *Meth. Enzym.* **99,** 387–402 (1983).
46. Hunter, T. & Sefton, B. M. *Proc. natn. Acad. Sci. U.S.A.* **77,** 1311–1315 (1980).
47. Booher, R. & Beach, D. *Molec. cell. Biol.* **6,** 3523–3550 (1986).
48. Kamps, M. P. & Sefton, B. M. *Oncogene* **2,** 305–315 (1988).
49. Gould, K. L. & Hunter, T. *Molec. cell. Biol.* **8,** 3345–3356 (1988).
50. Lipsich, L. A., Lewis, A. J. & Brugge, J. S. *J. Virol.* **48,** 352–360 (1983).

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Sequence of an unusually large protein implicated in regulation of myosin activity in *C. elegans*

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The *Caenorhabditis elegans* gene *unc-22* encodes a very large muscle protein, called twitchin, which consists of a protein kinase domain and several copies of two short motifs. The sequence of twitchin has unexpected similarities to the sequences of proteins of the immunoglobulin superfamily, cell adhesion molecules and vertebrate muscle proteins, including myosin light-chain kinase. These homologies, together with results from earlier genetic and molecular analyses, indicate that twitchin is involved in a novel mechanism of myosin regulation.

THE gene *unc-22* is one of more than 40 genes important for muscle assembly and function that have been identified in the nematode *Caenorhabditis elegans*[1]. Mutant animals that lack the *unc-22*-encoded protein (Unc-22) have a unique and puzzling phenotype: a nearly constant twitching of the body muscles. These muscles are unable to develop or sustain normal contractions. Instead, small regions within the myofilament lattice of individual muscle cells contract transiently in the absence of contraction of the adjacent lattice. Mutations are readily recovered and most of these are conditionally dominant[2,3]. All available extragenic suppressors of *unc-22* mutants are missense mutations of the main body wall myosin heavy-chain gene *unc-54*[4,5], indicating that the Unc-22 protein may act through myosin to regulate contraction. The *unc-22* gene has been cloned by transposon tagging[6] and this has permitted a molecular analysis of the gene[7].

The protein product of *unc-22*, which we call twitchin after the distinctive mutant phenotype, is located in the myosin-containing regions of muscle cells and has a relative molecular

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mass of ~600,000 (M_r 600 K)[7]. Several very large proteins of unknown function, such as titin[8-13], nebulin[14] and dystrophin[15], have been found in vertebrate muscle, but their relationships to twitchin are unclear. To understand the possible role of twitchin in muscle and to facilitate the search for homologous genes in other organisms, we determined the sequence of the *unc-22* gene.

The protein encoded by *unc-22*

The *unc-22* gene had been estimated to span more than 30 kilobases (kb), as deduced from DNA insertions and deletions that affect *unc-22* activity[7] (Fig. 1*a*). We sequenced a cosmid[16] and two adjacent restriction fragments to cover the entire region (Fig. 1*b*). The cosmid was sequenced by using a shotgun library prepared from total cosmid DNA, and the restriction fragment sequences were determined by conventional means. The resulting 47,081 base pairs (bp) of continuous sequence (GenBank EMBL accession number X15423) included the sites[17] of the two most extreme rearrangements (*st139* and *st185*; Fig. 1*a*), with 32,160 bp separating them.

The *unc-22* coding sequence in this genomic sequence was identified by using codon bias[18] and the consensus donor and acceptor sites of *C. elegans* introns[19]. The repeated nature of the encoded protein sequence (see below) also aided in the exon-intron assignments. Furthermore, complementary DNA sequence (Fig. 1*b*) confirmed the four 3'-most exons and also identified the poly(A) site (at position 34,386).

The coding sequence is divided among 14 exons, the largest of which is 9,613 bp. The 13 introns have the base composition and size typical of *C. elegans* introns[19]. The ATG at position 14,070 has tentatively been assigned as the initiator methionine

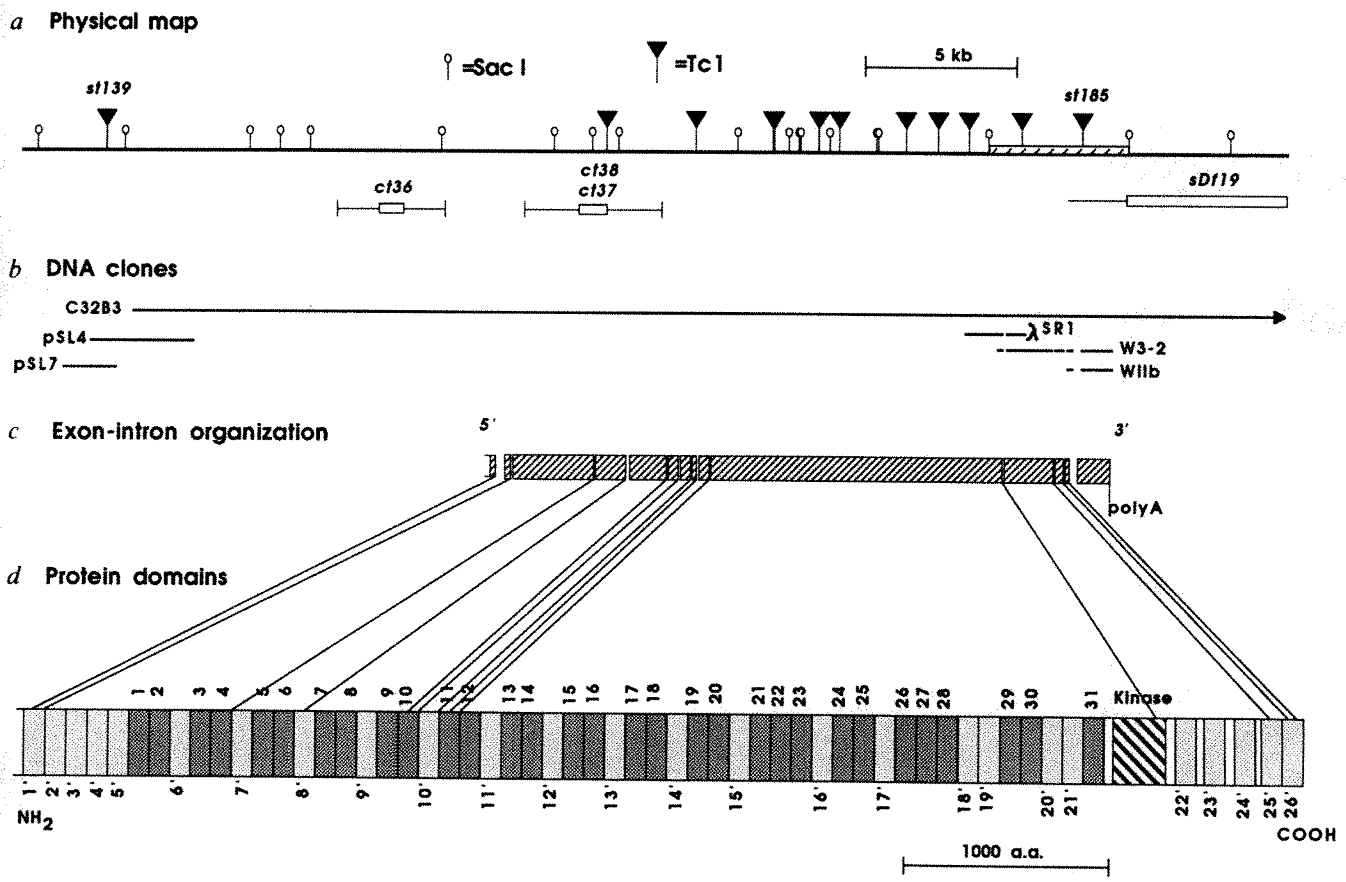

FIG. 1 The *unc-22* gene and its encoded polypeptide. *a*, Physical map of the *unc-22* region. The restriction map, the position of 12 transposon Tc*1* insertions that result in the *unc-22*-mutant phenotype, and the positions of the deficiencies *sDf19, ct36, ct37* and *ct38* are shown (modified from ref. 7). The position of the breakpoint of the deficiency *sDf19* orientates the physical map relative to the genetic map[6,36-38]. The open boxes represent the extent of deletions and the lines extending from them indicate uncertainties of position. The stippled box to the right indicates the 4.6-kb fragment used to probe the cDNA libraries. *b*, DNA clones that were sequenced. A portion of the cosmid clone C32B3, and two small overlapping plasmid genomic clones pSL4 and pSL7 are shown. LSR1, W3-2 and W11b on the right are cDNA clones. *c*, Exon-intron organization of the *unc-22* gene. The uncertaintiy in the 5' end of the transcript is denoted by the open box at the left. The polyadenylation addition site is indicated at the right. The scale is the same as in *a* and *b* and the gene is aligned with the physical map in *a*. *d*, Schematic representation of domains in the twitchin amino-acid sequence. The single protein kinase domain is denoted by the hatched box, the 31 copies of motif I are represented as darkly shaded boxes (1-31), and the 26 copies of motif II are represented as lightly shaded boxes (1'-26'). Small regions lacking homology either to themselves or to motifs I or II are indicated by the unshaded areas. Uncertainty in the translation start site is indicated by the open box at the left.

METHODS. A single shotgun M13 library was constructed[39] from the entire cosmid C32B3 (ref. 16). Dideoxy sequencing[40,41] reaction products from all clones were fractionated on polyacrylamide wedge-spacer gradient gels[42] and from one-third of the clones, on non-gradient gels for extended readings. Sequences were compiled and analysed with the programs of R. Staden[43]. After analysis of ~600 independent clones with ~1,050 gel readings, the sequence lay in 20 contigs. To close these gaps, thirty oligonucleotide primers (each 17-18 bases; synthesized by the Emory Microchemical Facility) were used to obtain a single contiguous sequence of 44,977 bp. To resolve ambiguities and read both strands of the cosmid insert we used 10 more oligonucleotide primers. The entire 47,081-bp sequence (including inserts of pSL4 and pSL7) was determined on both strands. Probable exons were determined by Staden's codon usage method, initially by using a codon table for abundantly expressed genes in *C. elegans*[18], and later using a codon table compiled from the *unc-22* gene itself. Candidate introns were detected by searching for consensus donor and acceptor sequences, and for conformity to the typical *C. elegans* intron[19]. The deduced amino-acid sequence was compared with others in the Protein Identification Resource database (release 20) and all possible reading frames of each GenBank DNA sequence (release 59) using the HOMOLOGY program of GENEPRO (Riverside Scientific Enterprises, Seattle, Washington).

codon. Although this is almost 13 kb from the *unc-22* (*st139*::Tc1) transposon insertion site (Fig. 1*c*), no likely exons could be found in the intervening region. The *st139* insertion could act at a distance or an unusually large intron could span the region. The TAA stop codon at 33,439 lies 66 bp downstream of the *st185* transposon insertion site and 948 bp upstream of the poly(A) site. We calculated the minimum message size to be 19,092 nucleotides, with a coding sequence of 18,147 bases. The predicted protein has 6,049 amino acids with an estimated M_r of 668,520.

Two ~100-amino-acid repeated motifs

Analysis of the predicted twitchin amino-acid sequence shows that most of the protein consists of two repeated motifs, each of ~100 amino acids (Fig. 1*d*). The most abundant motif, designated motif I, is present as 31 copies, arranged in 12 tandem pairs, 2 triplets and one singlet. The copies of motif I vary in length from 98–102 residues and are all rich in proline (~9%) (Fig. 2*a*). Comparison of all the copies against one another yields a consensus sequence in 55 of the positions scattered throughout the motif. Two regions particularly highly conserved between motif I copies are shown enclosed in boxes in Fig. 2*a*.

Each set of motif I copies is generally separated from the next set by a copy of a second motif, motif II, which varies in length from 91–95 residues. Motif II is repeated 26 times (Fig. 3*a*); the similarity of motif II copies to one another is less than that of motif I copies to one another, and less stringent criteria were used to derive a consensus sequence for motif II. One region of high conservation among motif II copies is boxed in Fig. 3*a*. Not all the copies of motif II are bracketed by motif I copies (Fig. 1*d*): five tandem copies of motif II occur in the N-terminal portion and five nearly tandem copies occur in the C-terminal portion of the polypeptide.

Five of the thirteen introns are positioned at the boundaries of motif I or motif II copies (introns 2, 3, 6, 7 and 9; see Fig. 1*c*). This is compatible with the notion that *unc-22* evolved by fusion of primordial genes containing either motif I or motif II, and then by duplication and divergence.

Similarity to myosin light-chain kinases

Searches of the PIR protein and GenBank DNA databases revealed that a C-terminal portion of twitchin devoid of motif I or II copies is homologous to the catalytic domains of protein kinases. The twitchin sequence has all 14 very highly conserved residues shared among all protein kinases[20]. The kinase portion of twitchin has greatest similarity to the kinase domain of the chicken smooth-muscle myosin light-chain kinase (csmMLCK)[21], no gaps are needed for the alignment of the two regions, with 52% identical residues over a stretch of 250 amino-acid residues (Fig. 4). The kinase portion of twitchin is also 44% identical (alignment without gaps) to the protein kinase domain of rabbit skeletal-muscle myosin light-chain kinase (rskMLCK; data not shown)[22]. Similar to csmMLCK and rskMLCK, twitchin has residues typical of serine/threonine kinases[20] (Fig. 4). Twitchin has no sequence homology to the calmodulin-binding domains of either csmMLCK (Fig. 4) or rskMLCK.

In addition to the homology between csmMLCK and twitchin in their kinase domains, we also found one copy of motif I and one-and-a-half copies of motif II in the published csmMLCK sequence (Figs 2*b*, 3*b* and 4). Both the order and spacing of motif I and motif II copies is conserved between the two sequences; recent cDNA-sequence data for csmMLCK indicates that

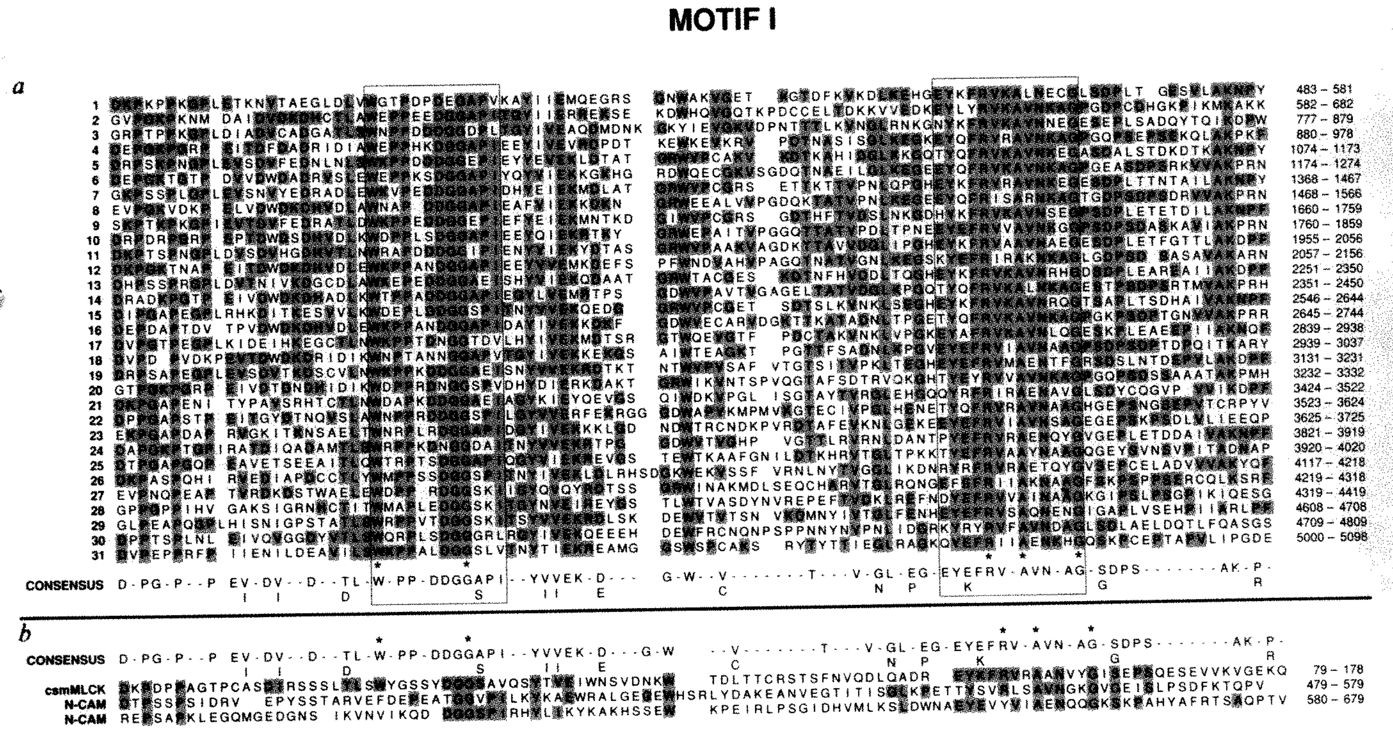

FIG. 2 Motif I (single-letter amino-acid code). *a*, The 31 copies of motif I. Numbers 1–31 on the left correspond to the darkly shaded boxes in Fig. 1*d*. The numbers on the right indicate positions in the twitchin deduced amino-acid sequence. Alignment was done by inspection. For each position, amino-acid residues found in at least 9 copies of motif I are shaded. The 55-residue consensus sequence is composed of residues that occur at corresponding positions in at least 14 out of 31 copies, or two residues that occur at corresponding positions between them in at least 19 out of 31 copies. Asterisks denote the five positions in which the same amino acid is found in all 31 copies. *b*, Similarity of motif I of twitchin to sequence in other proteins. The full copy of motif I in csmMLCK[21] is identical to the motif I consensus at 28 positions. For the two tandem copies of motif I in N-CAM[24], residues 479–578 are identical to the motif I consensus at 23 positions, and residues 580–673 are identical to the motif I consensus at 22 positions. Boxes enclose regions particularly conserved between motif-I copies.

the partial copy represents a complete copy of motif II and, indeed, yet another copy of motif II is upstream of that (N. Olson and A. R. Means, manuscript in preparation). By contrast, the rskMLCK lacks copies of either motifs I or II and its similarity to twitchin is limited to the protein kinase domain.

The high degree of similarity of twitchin with csmMLCK and rskMLCK in their kinase domains strongly indicates that one function of twitchin is to phosphorylate a muscle protein, presumably the regulatory myosin light chain. The extended similarity between twitchin and csmMLCK (37% identical and 47% similar allowing for conservative changes over a sequence of 644 residues in twitchin) indicates that these proteins possess additional conserved functions.

Motif II and immunoglobulin-like domains

The superfamily of proteins with sequence homology to immunoglobulins has been divided into three sets, the V set, the C1 set and the C2 set[23]. Motif II has homology to domains in the proteins of this superfamily, and greatest similarity to those in the proteins of the C2 set, a group that includes neural cell adhesion molecule (N-CAM), myelin associated glycoprotein (MAG), the receptor for platelet derived growth factor (PDGFR) and the Fc receptor (FcR) (Fig. 3*c*). The motif II consensus sequence matches 13 out of 25 residues in an immunoglobulin C2-set consensus sequence. The copies of twitchin motif II are more similar to one another than they are to other members of the immunoglobulin superfamily, presumably reflecting special functional constraints.

All previously recognized members of the immunoglobulin superfamily are cell surface or extracellular molecules, and yet twitchin (and csmMLCK) is totally intracellular. As might be expected for an intracellular molecule, the twitchin motif generally lacks the cysteines that form intra-chain disulphide bonds in immunoglobulin domains (copies 1′ and 20′ are the exception, in which both cysteines are found). The absence of these cysteines is not without precedent, because other members of the immunoglobulin superfamily have been found that lack the cysteines[23]. For these exceptions and in most copies of twitchin motif II, hydrophobic residues reside at the positions of the conserved cysteines.

Twitchin motifs and cell adhesion molecules

Motif I of twitchin is similar to two tandem segments in the neural cell adhesion molecule (N-CAM)[24] (residues 479-578 and residues 580-673), with many of the twitchin consensus residues present at a spacing similar to that in the N-CAM copies (Fig. 2*b*). These portions of NCAM have similarity to segments in other cell adhesion molecules of insects and mice[25,26]. This family of adhesion proteins has segments that are similar to the type III domains of fibronectin[25,27], as does twitchin motif I (especially over the last 33 residues of motif I). Twitchin, csmMLCK and these cell adhesion molecules contain both motifs I and II, but all other previously recognized members of the immunoglobulin superfamily have only motif II. It is striking that twitchin and the cell adhesion molecules N-CAM and fasciclin II each begin with five tandem copies of

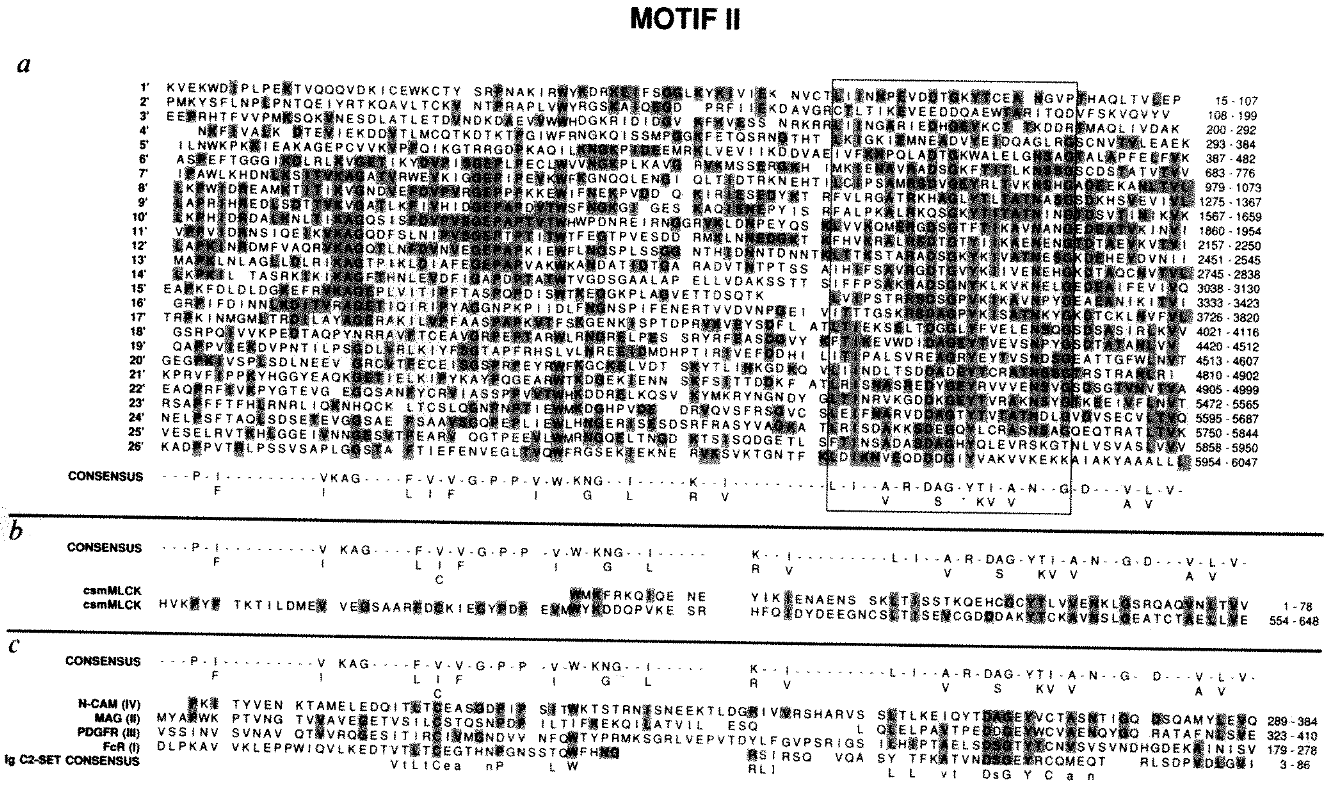

FIG. 3 Motif II (single-letter amino-acid code). *a*, The 26 copies of motif II in twitchin. Numbers 1′–26′ on the left correspond to the lightly shaded boxes in Fig. 1*d*. At each position, amino acids found in at least five copies are shaded. The consensus sequence was derived as residues present at corresponding positions in at least 9 out of 26 copies, or two residues that occur at corresponding positions between them at least 14 out of 26 copies. *b*, Similarity of motif II of twitchin to sequences in csmMLCK. Amino-acid residues identical to the consensus are shaded. The partial copy of motif II in csmMLCK matches the twitchin consensus at 15 positions and the complete copy of motif II in csmMLCK matches the twitchin consensus at 25 positions. *c*, The twitchin motif II consensus is compared with the following sequences of members of the C2 set of the immunoglobulin superfamily of proteins: N-CAM, fourth domain[24]; myelin-associated glycoprotein (MAG), second domain[44]; PDGFR, third domain[45], and the immunoglobulin Fc receptor (FcR) first domain[46]. Residues identical to the twitchin consensus are shaded. Also displayed is a consensus for the immunoglobulin C2-set domains (modified from ref. 23). Upper-case letters denote the most strongly conserved amino acids, and the lower-case letters denote the less strongly conserved residues at various positions. A box encloses one region particularly highly conserved between motif-II copies.

Most probably twitchin associates with the thick filament, as it is found in the myosin-containing A bands and co-localizes with myosin in mutants in which muscle structure is abnormal[7]. The regular spacing and pattern of the motifs throughout much of the protein are well suited for the interaction with the repeated structure of the thick filament, which consists of a regular staggered array of myosin rods and paramyosin. Furthermore, both the myosin rod and paramyosin are composed of tandem copies of a 28-amino-acid motif estimated to be 42-Å long[33,34], a size similar to that of an immunoglobulin domain and perhaps motif II as well. But the regularity of the motifs is disrupted in several places, including two occurrences of motif-I triplets, one instance of a motif-I singlet, and two occurrences of motif II doublets (Fig. 1*d*). Whether these represent adaptations to fixed irregularities in thick filament structure or relaxed functional requirements of the protein is unclear; note that deletions in the gene that maintain the reading frame, yet disrupt the domain structure, can result in proteins with nearly normal function[35].

The association of the protein with the thick filament may localize the kinase domain near the myosin heads on the surface of the filament. This also means, however, that neither the kinase nor its substrate myosin can diffuse within the cell. Yet each kinase domain must act on many myosins, as there is estimated to be only one mole of twitchin per 10–50 moles of myosin. Perhaps the kinase domain has enough flexibility to allow it to act on many myosins. Alternatively, the repeated domains themselves could play a part in regulation. If, indeed, the protein interacts along the long axis of the filament, it could serve to coordinate the activity of many myosins, in a manner analogous to the way in which tropomyosin regulates multiple actins. Also, like tropomyosin, the molecule could propagate changes in activity generated by the kinase domain down the filament, or calcium levels could directly alter the conformation of the repetitive part of the molecule. Consistent with these ideas is the finding that twitchin is required in near stoichiometric amounts; the loss of just half of the *unc-22* activity results in detectable twitching.

Does twitchin also have a fundamental role in thick filament assembly? The myofilament lattice is disrupted in nearly all *unc-22* mutants, but the numbers of thick filaments, even in the complete absence of twitchin, are about normal[2]. Furthermore, the assembly of these filaments into a sarcomere can be normal in the absence of twitchin, provided that certain missense mutations are present in the myosin head region[4,5]. Twitchin seems, therefore, to be unnecessary for either normal thick-filament formation or normal sarcomere assembly; a structural role for twitchin may yet, however, be found. We suggest instead that this unusually large protein has an important role in regulating the contraction–relaxation cycle. □

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1. Waterston, R. H. in *The Nematode* Caenorhabditis elegans (ed. Wood, W.B.) 281–335 (Cold Spring Harbor Laboratory, New York, 1988).
2. Waterston, R. H., Thomson, J. N. & Brenner, S. *Devl Biol.* **77**, 271–302 (1980).
3. Moerman, D. G. thesis, Simon Fraser Univ. Burnaby, British Columbia (1980).
4. Moerman, D. G., Plurad, S., Waterston, R. H. & Baillie, D. L. *Cell* **29**, 773–781 (1982).
5. Dibb, N. J. *et al. J. molec Biol.* **183**, 543–551 (1985).
6. Moerman, D. G., Benian, G. M. & Waterston, R. H. *Proc. natn. Acad. Sci. U.S.A.* **83**, 2579–2583 (1986).
7. Moerman, D. G., Benian, G. M., Barstead, R. J., Schriefer, L. A. & Waterston, R. H. *Genes Dev.* **2**, 93–105 (1988).
8. Maruyama, K., Kimura, S., Yoshidomi, H., Sawada, H. & Kikuchi, M. *J. Biochem.* **95**, 1423–1433 (1984).
9. Kurzban, G. P. & Wang, K. *Biochem. biophys. Res. Commun.* **150**, 1155–1161 (1988).
10. Wang, K., McClure, J. & Tu, A. *Proc. natn. Acad. Sci. U.S.A.* **76**, 3698–3702 (1979).
11. Whiting, A., Wardale, J. & Trinick, J. *J. molec. Biol.* **205**, 263–268 (1989).
12. Wang, K., Ramirez-Mitchell, R. & Palter, D. *Proc. natn. Acad. Sci. U.S.A.* **81**, 3685–3689 (1984).
13. Trinick, J., Knight, P. & Whiting, A. *J. molec. Biol.* **180**, 331–356 (1984).
14. Wang, K. & Williamson, C. L. *Proc. natn. Acad. Sci. U.S.A.* **77**, 3254–3258 (1980).
15. Koenig, M., Monaco, A. P., & Kunkel, L. M. *Cell* **53**, 219–228 (1988).
16. Coulson, A., Sulston, J., Brenner, S. & Karn, J. *Proc. natn. Acad. Sci. U.S.A.* **83**, 7821–7825 (1986).
17. Mori, I., Benian, G. M., Moerman, D. G. & Waterston, R. H. *Proc. natn. Acad. Sci. U.S.A.* **85**, 861–864 (1988).
18. Emmons, S. W. in *The Nematode* Caenorhabditis elegans (ed. Wood, W. B.) 57–59 (Cold Spring Harbor Laboratory, New York, 1988).
19. Blumenthal, T. & Thomas, J. *Trends Genet.* **4**, 305–308 (1988).
20. Hanks, S. K., Quinn, A. M. & Hunter, T. *Science* **241**, 42–52 (1988).
21. Guerriero, V., Russo, M. A., Olson, N. J., Putkey, J. A. & Means, A. R. *Biochemistry* **25**, 8372–8381 (1986).
22. Takio, K., Blumenthal, D. K., Walsh, K. A., Titani, K. & Krebs, E. G. *Biochemistry* **25**, 8049–8057 (1986).
23. Williams, A. F. & Barclay, A. N. *A. Rev. Immun.* **6**, 381–405 (1988).
24. Cunningham, B. A. *et al. Science* **236**, 799–806 (1987).
25. Harrelson, A. L. & Goodman, C. S. *Science* **242**, 700–708 (1988).
26. Moos, M. *et al. Nature* **334**, 701–703 (1988).
27. Kornblihtt, A. R., Umezawa, K., Vibe-Pedersen, K. & Baralle, F. E. *EMBO J.* **4**, 1755–1759 (1985).
28. Schiffer, M., Girling, R. L., Ely, K. R. & Edmundson, A. B. *Biochemistry* **12**, 4620–4631 (1973).
29. Edelman, A. M., Blumenthal, D. K. & Krebs, E. G. *A. Rev. Biochem.* **56**, 567–613 (1987).
30. Kamm, K. E. & Stull, J. T. *A. Rev. Pharmacol. Tox.* **25**, 593–620 (1985).
31. Harris, H. E., Tso, M.-Y. W. & Epstein, H. F. *Biochemistry* **16**, 859–865 (1977).
32. Lehman, W. & Szent-Gyorgyi, A. G. *J. gen. Physiol.* **66**, 1–30 (1975).
33. McLachlan, A. D. & Karn, J. *Nature* **299**, 226–231 (1982).
34. Kagawa, H., Gengyo, K., McLachlan, A. D., Brenner, S. & Karn, J. *J. molec. Biol.* **207**, 311–333 (1989).
35. Kiff, J. E., Moerman, D. G., Schriefer, L. A. & Waterston, R. H. *Nature* **331**, 631–633 (1988).
36. Moerman, D. G. & Baillie, D. L. *Genetics* **91**, 95–103 (1979).
37. Moerman, D. G. & Waterston, R. H. *Genetics* **108**, 859–877 (1984).
38. Rogalski, T. M. & Baillie, D. L. *Molec. Gen. Genet.* **201**, 409–414 (1985).
39. Bankier, A. T. & Barrell, B. G. in *Techniques in the Life Sciences* Vol. B508 (ed. Flavell, R. A.) 1–34 (Elsevier, Ireland, 1983).
40. Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5463–5467 (1977).
41. Tabor, S. & Richardson, C. C. *Proc. natn. Acad. Sci. U.S.A.* **84**, 4767–4771 (1987).
42. Ansorge, W. & Labeit, S. *J. Biochem. biophys. Meth.* **10**, 237–243 (1984).
43. Staden, R. *Nucleic Acids Res.* **14**, 217–231 (1986).
44. Arquint, M. et al. *Proc. Acad. Sci. U.S.A.* **84**, 600–604 (1987).
45. Yarden, Y. *et al. Nature* **323**, 226–232 (1986).
46. Lewis, V. A., Koch, T., Plutner, H. & Mellman, I. *Nature* **324**, 372–375 (1986).
47. Needleman, S. B. & Wunsch, D. C. *J. molec. Biol.* **48**, 545–552 (1970).

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Is Cygnus X-3 blowing bubbles?

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CYGNUS X-3, one of the brightest X-ray sources in the Galaxy and perhaps also a source of very-high-energy (TeV) photons or particles, is thought to be a close binary system (orbital separation $\sim 10^{11}$ cm and period 4.8 h) whose emission is powered by accretion of mass from a main-sequence star like the Sun onto a neutron star, or possibly a black hole. The X-ray luminosity, $L_x \sim 10^{38}$ erg s^{-1}, implies a radiation rate near the Eddington limit. Recent radio observations of Cyg X-3 (ref. 1) have revealed extended radio lobes on a scale of ~0.3 pc, elongated in the same direction in which clouds of relativistic plasma, discovered by very-long-baseline interferometry observations[2-4] during radio outbursts, are ejected from the binary system. I argue here that the extended radio lobes can be understood as bubbles blown into a dense interstellar medium by the cumulative energy of the bursts from the central object. If this is correct, the extended lobes of Cyg X-3 would have to be ~2,000 years old.

At radio frequencies, Cyg X-3 shows strong, irregularly spaced bursts of emission in which the flux rises by a factor of 100–1,000 above the typical level[5]. The mean interval between bursts is $p_b \sim 120$ days. The bursts can be interpreted as synchrotron radiation from an expanding cloud of relativistic electrons in a magnetic field of 10^{-2} G. Very-long-baseline interferometry (VLBI) observations[2-4] during bursts show an expanding elongated radio-frequency source on a scale of 10^{14} cm. Assuming a distance to Cyg X-3 of 10 kpc, the projected expansion velocity is $V_{exp} \approx 0.3$–$0.4\ c$. The minimum energy per burst, U_b, required to power the emission is about 10^{44} erg (ref. 7), and the associated average luminosity L_b is $U_b/p_b \approx 10^{37}$ erg s^{-1}, which is about 0.1 L_x. The recently discovered[1] extended radio lobes have a linear extent of $D \approx 10^{18}$ cm. The extended lobes are elongated in the direction of the VLBI-detected expansion, which suggests a causal connection with the bursts.

The physical properties of the extended lobes calculated assuming that the radio emission is synchrotron radiation are summarized in Table 1. The minimum energy, U_{min}, stored by relativistic electrons and magnetic fields in the lobes is estimated to be $\sim 10^{45}$ erg. This energy could be supplied by the bursts in a time $t_{min} \approx U_{min}/L_b \approx 3$ yr. This energy could be supplied by the bursts in a time $t_{min} \approx U_{min}/L_b \approx 3$ yr. This is of the same order of magnitude as the dynamical timescale $t_{dyn} \approx D/V_{exp}$ derived from the VLBI-detected source expansion. In contrast, the radiation-loss time of the synchrotron electrons, t_{loss}, is $\sim 10^6$ yr. The fact that $t_{loss} \gg t_{dyn}$, t_{min} suggests that most of the energy supplied to the lobes by the central source is expended in driving the expansion of the lobes, rather than in building up the internal energy density and compensating for synchrotron losses. In that case, the lobes must be much older than the simple estimate t_{min} would suggest.

I propose a model for the extended lobes that is analogous to the formation of bubbles in the interstellar medium by stellar winds. It differs from conventional models of radio-lobe formation used in the context of active galaxies and quasars in the assumption that the momentum flux of the outflow is not well collimated as in a jet with constant cross-section, but instead is distributed over an area scaling as D^2, where D is the linear extent of the source. This would be the case for instance in a conical jet with a fairly large constant opening angle, or in a quasi-spherical expansion.

On timescales $t \gg p_b$ the expansion is driven by the cumulative energy of the bursts. A self-similar solution describes a shock propagating into the interstellar medium with typical radius[8] $R(t) = \alpha(L_b/\rho)^{1/5} t^{3/5}$, where ρ is the density of the interstellar medium, t is the age of the source and α is a numerical constant of the order of unity. The hot interior of the bubble and the shocked interstellar medium are separated by a contact discontinuity at radius $R_{cd} \approx 0.83\ R(t)$. For spherical symmetry (opening angle near 4π), $\alpha \approx 0.88$ during the initial adiabatic phase of the expansion and $\alpha \approx 0.76$ after the shell of swept-up interstellar medium has collapsed through internal cooling and radiates efficiently. The detailed shape of the lobe depends on the (unknown) angular distribution of the momentum flux driving the expansion, and can be calculated using a generalized Kompaneets approximation[9,10]. The lobes will be elongated along the direction of the beamed outflow from the central source, as observed.

Given the observed size, D, of the extended lobes, one can use the above expansion law to estimate the age of the source:

$$t\,(\text{yr}) \approx 1.5 \times 10^3 \alpha^{-5/3} D_{18}^{5/3} n_2^{1/3} L_{37}^{-1/3} \tag{1}$$

where D_{18} is the linear size of the source in units of 10^{18} cm. n_2 is the number density in the interstellar medium in units of 100 cm^{-3} and L_{37} is the driving luminosity in units of 10^{37} erg s^{-1}. The age of a few thousand years for the extended source is much less than the evolutionary timescale for a close binary system ($\geqslant 10^6$ yr). This suggests that the bursts driving the expansion are a fairly recent phenomenon in Cygnus X-3. The expansion velocity of the lobes is $\dot{D} = 1.3 \times 10^2 \alpha^{8/3} L_{37}^{1/3} n_2^{-1/3} D_{18}^{-2/3}$ km s^{-1}, and the internal pressure in the lobes is roughly equal to the pressure behind the strong shock, $P_s \approx (3/4)\rho\dot{D}^2 \approx 2 \times 10^{-8} \alpha^{16/3} n_2^{1/3} L_{37}^{2/3} D_{18}^{-4/3}$ erg cm^{-3}. This yields a total thermal energy U_{th} of the lobes of the order of $(3/2)P_s \times (4\pi D^3/3) \approx 10^{47} \alpha^{16/3} L_{37}^{2/3} n_2^{1/3} D_{18}^{5/3}$ erg. Comparing U_{th} with the minimum energy U_{min} needed to explain the extended synchrotron emission, I estimate that ~1% of the internal energy must reside in the relativistic electrons and magnetic fields responsible for the synchrotron emission. The total energy U_{tot} supplied to the lobes, including the kinetic energy of the swept-up interstellar material, is $L_b t = 4.5 \times 10^{47} \alpha^{-5/3} D_{18}^{5/3} L_{37}^{2/3} n_2^{1/3}$ erg $\approx 4.5 \times 10^2 \alpha^{-5/3} U_{min}$.

In its lifetime the extended source has swept up interstellar gas with total mass $M_s(\text{g}) \approx (4\pi/3)\rho D^3 \approx 10^{33} n_2 D_{18}^3$. The swept-up gas directly behind the shock has a temperature $T_+\,(\text{K}) \approx (3\mu m_H/16 k_B)\dot{D}^2 = 5 \times 10^5 \alpha^{16/3} L_{37}^{2/3} n_2^{-2/3} D_{18}^{-4/3}$, where $\mu \approx 1.4$ is the mean relative molecular mass per hydrogen atom, m_H is the mass of a hydrogen atom and k_B is the Boltzmann constant. Radiation losses per unit volume $C(T)$ in this hot gas of ionized hydrogen and multiply ionized other elements are $n_e n_p \Lambda(T)$, where the cooling function $\Lambda(T) \approx 1.6 \times 10^{-22} T_6^{-0.6}$ erg cm^3 s^{-1}, T_6 being the temperature of the gas in units of 10^6 K. Here n_e and n_p are the electron and proton densities. Cooling is primarily due to line emission for $T \leqslant 10^7$ K. Defining a net compression ratio $n_s/n \equiv r$ for the swept-up gas and taking $n_e \approx n_p \approx n_s$, the typical cooling time behind the shock, $t_C \approx 3 n_s k_B T/(2C(T)) \approx 1.5 \times 10^2 (T)_6^{1.7} r^{-1} n_2^{-1}$ yr. This is shorter than the age of the lobes provided that $rn_2 \geqslant 0.1$, so that it is likely that the shocked interstellar material has collapsed into a thin shell, or is in the process of doing so.

The energy advected into the shell across the shock per unit time, $L_s \approx 4\pi D^2 \times (\rho\dot{D}^3/2) \approx 2\pi\alpha^5(3/5)^3 L_b \equiv \varepsilon L_b$, is largely radiated away ($\varepsilon \approx 0.3$ if $\alpha \approx 0.76$, and L_s is the luminosity of the shocked material). The hot layer of ionized hydrogen and multiply ionized ions cools by radiating most of its energy in the ultraviolet at wavelengths <912 Å. At a column density of $N \approx 10^{17}$ cm^2 behind the shock, the temperature reaches $T \approx 10^4$ K and a recombination zone begins which reabsorbs this ionizing ultraviolet radiation. This zone therefore radiates with a total luminosity of about L_s, cooling through optical and ultraviolet radiation[11,12]. A typical cooling rate has $\Lambda_{rec} \approx 2 \times 10^{-23}$ erg cm^3 s^{-1} (refs 13, 14). The thermal free-free emission from the dense shell comes primarily from the recombination zone. The free-free emissivity per unit volume is $n_e n_p \Lambda_{ff}(T)$, with a free-free cooling function $\Delta_{ff}(T) \approx 2.4 \times 10^{-25} T_4^{1/2}$ erg cm^3 s^{-1}, where T_4 is the temperature of the recombination zone in units of 10^4 K. The total free-free luminosity can be estimated as $L_{\mathrm{ff}} \approx \Lambda_{\mathrm{ff}}(T)\varepsilon L_{\mathrm{b}}/(\Lambda_{\mathrm{rec}} + \Lambda_{\mathrm{ff}}(T)) \approx 4\times 10^{34} T_4^{1/2}(\varepsilon/0.3)L_{37}$ erg s^{-1}. The free-free emission from the hot region directly behind the shock is about a factor of 100 less intense. At low freqencies ($h\nu \ll k_{\mathrm{B}}T$) the monochromatic free-free emission flux $(S_\nu)_{\mathrm{ff}}$ at the Earth for an assumed distance of $d = 10$ kpc is about $(h/k_{\mathrm{B}}T)(L_{\mathrm{ff}}/4\pi d^2) \approx 2(\varepsilon/0.3)L_{37}$ mJy. Extinction can be neglected at a wavelength of 6 cm. This is about a factor of two less than the observed extended flux density. It therefore seems likely that a significant part of the radio emission is synchrotron radiation, as was assumed by Strom *et al.*[1]. This interpretation is supported by their detection of (partial) polarization of the 6-cm emission. The relative contribution of thermal free-free radiation will remain uncertain until multi-frequency observations determine the spectrum of the radio emission from the extended lobes.

The relativistic electrons filling the lobes have probably not been produced directly by the bursts. A power-law distribution of relativistic electrons—$(\mathrm{d}N/\mathrm{d}\gamma) \propto \gamma^{-s}$ where γ is the Lorentz factor (integrated over volume)—that are created at an individual burst in a volume V_0, carrying a fraction f of the total burst energy U_{b}, contributes a relativistic-electron energy $U_{\mathrm{e}} = fU_{\mathrm{b}}(V/V_0)^{-(s-1)/3}$ after adiabatic expansion into the volume V of the extended lobes. The VLBI-detected clouds have a volume $V_0 \leqslant 10^{45}\ \mathrm{cm}^3$, whereas the observed spectrum is consistent with[4,7] $s \approx 2$. After expansion into the whole lobe volume $V \approx 10^{54}\ \mathrm{cm}^3$, the remaining relativistic-electron energy per burst is $U_{\mathrm{e}} \leqslant 10^{-3} fU_{\mathrm{b}} \approx 10^{41} f(U_{\mathrm{b}})_{44}$ erg (where $(U_{\mathrm{b}})_{44}$ is the burst energy in units of 10^{44} erg). This is four orders of magnitude less than the relativistic-electron energy present in the lobes if the observed 6-cm emission has a significant non-thermal component. This excludes the possibility that the extended lobes are adiabatically expanding, optically thin synchrotron clouds created by a single large burst in the central object about three years ago. The energy of the cloud accumulated from all the bursts in a time $t_{\mathrm{loss}} > t \gg p_{\mathrm{b}}$ is $U_{\mathrm{c}} \approx (t/p_{\mathrm{b}})U_{\mathrm{e}} = f(V/V_0)^{-(s-1)/3}L_{\mathrm{b}}t = 10^{-3}fU_{\mathrm{tot}}$. For f of the order of unity, $U_{\mathrm{c}} \approx U_{\mathrm{min}}$, but it seems unlikely that all the energy in the bursts resides in relativistic electrons.

The total monochromatic synchrotron luminosity of the lobes that is due to the accumulated relativistic electrons is $S_\nu = (t/p)(B/B_0)^{(s+1)/2}(V/V_0)^{-(s-1)/3}S_\nu(\mathrm{burst})$. The observations of the bursts are consistent with $B_0 \approx 0.1$ G, whereas the lobes have $B_{\mathrm{eq}} \approx 10^{-4}$ G. Here B_0, B and B_{eq} are the magnetic field in the VLBI clouds, the extended lobes and the equipartition field respectively. This gives $S_\nu \approx 10^{-4}S_\nu(\mathrm{burst}) \leqslant 1$ mJy, as the strongest bursts have $S_\nu(\mathrm{burst}) \approx 10$ Jy. This is at least a factor of five less than the observed flux density (see Table 1). Some parameters, however, are quite uncertain—in particular the energy density in the lobes and the magnetic field strength B, which are derived from the minimum-energy assumption. These estimates imply that some reacceleration and/or fresh production of relativistic electrons is required in the lobes to partly compensate for expansion losses, which increases the synchrotron luminosity.

An efficient acceleration mechanism that is likely to operate in the lobes is diffusive shock acceleration[15]. Particles will be accelerated at the strong exterior shock that encloses the lobes or at shocks generated when clouds formed at each burst collide with the dense shell of shocked interstellar material behind that exterior shock. The mean energy gain in this process of a particle with Lorentz factor γ, diffusing with spatial diffusion coefficient K, at a strong shock with propagation velocity u_{s} is

$$\frac{1}{\gamma}\left(\frac{\mathrm{d}\gamma}{\mathrm{d}t}\right) \approx \frac{u_{\mathrm{s}}^2}{4\langle K\rangle} \tag{2}$$

TABLE 1 Observational parameters

Distance Cyg X-3: 10 ± 2 kpc.
X-ray luminosity: $L_{\mathrm{X}} = 10^{38}\ \mathrm{erg\ s^{-1}}$
Mean 6-cm flux density associated with burst: $S_\nu(\mathrm{burst}) \approx 5$ Jy
Extended 6-cm flux density at Earth: $S(\nu = 4.9\ \mathrm{Ghz}) = 4.5$ mJy
Radio luminosity of extended lobes: $L_{\mathrm{r}} = 3\times 10^{30}\ \mathrm{erg\ s^{-1}}$
Diameter of extended lobes: $D = 0.3\ \mathrm{pc} = 10^{18}$ cm
Minimum energy: $U_{\mathrm{min}} = 10^{45}$ erg
Equipartition field: $B_{\mathrm{eq}} = 10^{-4}$ G

Here $\langle K\rangle$ is a mean spatial diffusion coefficient, defined by $\langle K\rangle = K_- + 4K_+$. The subscripts $-$ and $+$ denote typical values in the medium upstream and downstream of the shock. The relativistic electrons diffuse by gyro-resonant scattering from magneto-hydrodynamic waves. The associated diffusion coefficient is $K = (cr_{\mathrm{g}}/3)\ I(k_{\mathrm{res}})^{-2}$, where $r_{\mathrm{g}} = \gamma m_{\mathrm{e}}c^2/eB = 1.6\times 10^7\gamma B_{-4}^{-1}$ cm is the gyroradius of an electron with Lorentz factor factor γ and $I(k_{\mathrm{res}}) \equiv \delta B(k_{\mathrm{res}})/B$ is the relative magnetic amplitude of the waves at the resonant wavenumber $k_{\mathrm{res}} \approx 1/r_{\mathrm{g}}$. For strong turbulence ($\delta B(k)/B \approx 1$ at all scales), one has $K = K_0\gamma$, where $K_0 = m_{\mathrm{e}}c^3/3eB \approx 10^{18}B_{-4}^{-1}\ \mathrm{cm^2\ s^{-1}}$ is the diffusion coefficient of a particle with $\gamma \approx 1$. Electrons injected into the acceleration region with $\gamma \approx 1$ gain energy according to $\gamma(t) \approx 1 + u_{\mathrm{s}}^2t/(4\langle K_0\rangle)$. This means that electrons radiating by the synchoton mechanism at $\lambda = 6$ cm with a shock Lorentz factor $\gamma_{\mathrm{s}} \approx 1{,}000$ can be accelerated in a time $t_{\mathrm{acc}} \approx 8\gamma_{\mathrm{s}}K_0/u_{\mathrm{s}}^2 \approx 10^6(K_0)_{18}/(u_{\mathrm{s}})_8^2$ s, where I have assumed that $\langle K_0\rangle \approx 2K_0$ ($(u_{\mathrm{s}})_8$ is the shock propagation velocity in units of $10^8\ \mathrm{cm\ s^{-1}}$ and $(K_0)_{18}$ is the spatial diffusion coefficient in units of $10^{18}\ \mathrm{cm^2\ s^{-1}}$). Acceleration at the outer shock with $(u_{\mathrm{s}})_8 \approx 0.1$ can proceed to the required energies in $\sim 3(K_0)_{18}$ yr. This is much less than the age of the source provided $K_0 \leqslant 10^{21}\ \mathrm{cm^2\ s^{-1}}$, allowing for less-efficient scattering by weak turbulence with $\delta B(k_{\mathrm{res}})/B \approx 0.03$. Alternatively, if the central source ejects clouds moving ballistically, as does SS433[16], these clouds will collide with the outer dense shell of the lobes. This will also result in the formation of shocks when these individual clouds break up. With an assumed cloud velocity $V_{\mathrm{c}} \approx V_{\mathrm{exp}} \approx 0.35c$ and a cloud size r_{c}, the maximum time available for acceleration at such a collision is $r_{\mathrm{c}}/V_{\mathrm{c}} \approx 10^6(r_{\mathrm{c}})_{16}$ s (where $(r_{\mathrm{c}})_{16}$ is r_{c} in units of 10^{16} cm). As u_{s} is expected to be of the order of $V_{\mathrm{exp}} \sim 10^{10}\ \mathrm{cm\ s^{-1}}$, this is more than enough to (re)accelerate the electrons to the required GeV energies. It therefore seems likely that the modest amount of reacceleration required in this model can be supplied by diffusive shock acceleration, even with a low level of turbulence $\delta B(k_{\mathrm{res}})/B \approx 0.1$. The maximum energy that can be attained by electrons from continuously operating shock acceleration at the outer shock is determined by synchrotron losses. Writing these losses as $(\mathrm{d}\gamma/\mathrm{d}t)_{\mathrm{synch}} = -\gamma^2/\tau$, with $\tau = 6\pi m_{\mathrm{e}}c/\sigma_T B^2 = 2\times 10^9\ B_{-4}^{-2}$ yr (here σ_{T} the Thomson cross-section) and balancing them with the energy gain that is due to shock acceleration (equation (2)) (assuming as before that $\langle K\rangle = 2K_0\gamma$) I obtain $\gamma_{\mathrm{max}} \approx (u_{\mathrm{s}}^2\tau/8K_0)^{1/2} \approx 10^7(u_{\mathrm{s}})_8 B_{-4}^{-1}(K_0)_{18}^{-1/2})$. Expansion losses in the self-similar flow $(\mathrm{d}\gamma/\mathrm{d}t)_{\mathrm{exp}} \approx -(\dot{D}/D)\gamma \approx -3\gamma/5t$ become important at $\gamma \approx 5u_{\mathrm{s}}^2t/24K_0 \approx 10^8(u_{\mathrm{s}})_8^2\alpha^{-5/3}D_{18}^{5/3}n_2^{1/3}L_{37}^{-1/3}(K_0)_{18}^{-1}$. □

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1. Strom, R. G., van Paradijs, J. & van der Klis, M. *Nature* **337**, 234–236 (1989).
2. Geldzahler, B. J. *et al. Astropys. J.* **273,** L65–L69 (1983).
3. Spencer, R. E. & Johnston, K. J. in *RS Ophiuchi* (1985) *and the Recurrent Nova Phenomenon* (ed. Bode, F.) 215–219 (VNU Science Press, Utrecht, 1986).
4. Molnar, L. A., Reid, M. J. & Grindley, J. E. *Astrophys. J.* **331,** 494–508 (1988).
5. Bonnet-Bideaud, J. M. & Chardin, G. *Phys. Rep.* **170,** 325–404 (1988).
6. Johnston, K. J. *et al. Astrophys. J.* **309,** 707–713 (1986).
7. Seaquist, E. R. *Astrophys. J.* **207,** 88–95 (1976).
8. Weaver, R., McCray, R., Castor, J., Shapiro, P. & Moore, R. *Astrophys. J.* **218,** 377–395 (1977).
9. Icke, V. *Astr. Astrophys.* **202,** 177–188 (1988).
10. Bisnovatyi-Kogan, G. S. & Blinnikov, S. I. *Soviet Astr.* **26,** 530–537 (1983).
11. Curiel, S., Cantó y Luis, J. & Rodriguez, L. F. *Rev. Mex. Astr. Astrofis.* **14,** 595–602 (1987).
12. Cox, D. P. & Raymond, J. C. *Astrophys. J.* **298,** 651–659 (1985).
13. Shull, J. M. & McKee, C. F. *Astrophys. J.* **227,** 131–149 (1979).
14. Hartigan, P., Raymond, J. & Hartmann, L. *Astrophys. J.* **316,** 323–348 (1987).
15. Blandford, R. D. & Eichler, D. *Phys. Rep.* **154,** 1–75 (1987).
16. Vermeulen, R. C., Schillizzi, P. T., Icke, V., Fejes, I. & Spencer, R. E. *Nature* **328,** 309–313 (1987).

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Ultra-low-frequency variability in a simple atmospheric circulation model

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To assess the inherent variability of atmospheric flow, we have carried out extended runs of a primitive-equation model of the global troposphere. The longest-period forcing that we explicitly included was the annual cycle, yet the largest-scale structures in the flow were variable on much longer timescales, with maximum variability on periods of 10–40 years. This variability was generated internally by the nonlinear dynamics of the atmospheric flow. Forcings by slow variations of such quantities as sea surface temperature or incoming solar radiation were excluded. The results show that apparent correlations with the sunspot cycle[1–3] could be spurious. They also indicate that attempts to monitor systematic climate change require circulation statistics for periods longer than 40 years, and that studies of climate sensitivity based on global circulation models require integrations of at least that length.

The observed variability of atmospheric flow at low frequencies may be due either to the inherent, internally generated unsteadiness of that flow, or to external influences in the form of varying boundary forcing, changing solar radiation or to changes of atmospheric composition. The relative contribution of internal and external sources of variability is still uncertain. Arguments based on linear theories of instability and on the known limits of deterministic predictability suggest that internal variability dominates for periods less than 10–20 days. It is frequently assumed that external causes must be sought for lower-frequency variations. Certainly there has been a degree of success in relating part of the observed interannual variance to slowly varying patterns of sea surface temperature. The nonlinearity of atmospheric flow does not necessarily mean, however, that its behaviour is completely random on the longer timescale. Coherent and relatively long-lived structures can arise. Extreme regularity in highly nonlinear flow is well known in experiments on rotating convecting fluids[4], and long-lived coherent blocking patterns are observed in the atmosphere[5]. Here we show that internally generated variations of much lower frequencies than have previously been assumed dominate the spectrum of planetary-scale structures in an atmospheric model. The results imply that a degree of regularity underlies the apparently random fluctuations of the circulation.

We used a global version of the spectral primitive-equation model described in ref. 6. We used five equal mass layers in the vertical. The smallest horizontal scale included was equivalent to 21 wavelengths around the globe (wavenumber 21); this implies a smallest resolved scale of ~700 km, and is similar to the resolution of many operational global-circulation models. The model handles the nonlinear dynamics of the atmosphere in a relatively sophisticated manner, representing the large-scale circulation systems of both the tropics and mid-latitudes explicitly.

We modelled the effects of diabatic heating and friction by simple linear terms so that we could carry out extremely long integrations at comparatively modest cost. The atmospheric circulation was driven by a term in the thermodynamic equation that relaxed the temperature towards a 'radiative–convective equilibrium' on a timescale of 15 days. The solstice distribution of the equilibrium temperature is shown in Fig. 1*a*. Such a state is unstable, particularly to baroclinic instabilities in the middle latitudes which convert potential energy associated with the meridional temperature gradients into the kinetic energy of the atmospheric circulation. This energy conversion is eventually balanced by the destruction of kinetic energy by friction at the Earth's surface. We modelled friction by a simple 'Rayleigh drag law' in the lowest model layer. A force $-\mathbf{v}/\tau_d$ acts on the air in the lowest layer, where $\mathbf{v}$ is the horizontal wind vector and τ_d a timescale, which we chose to be one day. We varied the pole-pole radiative equilibrium temperature difference sinusoidally to represent the seasonal cycle. The model included no moisture (except implicitly, by the definition of the radiative-convective equilibrium state) and the lower surface was uniform with no mountains or heat sources and sinks.

The mean temperature structure at the solstices is shown in Fig. 1*b*. The atmospheric circulation has increased the static stability and reduced the meridional temperature gradients by transporting heat upwards and polewards. Further discussion of the mean global circulation of this model and of its sensitivity to the imposed parameters is given in ref. 6.

The variability of the large-scale atmospheric flow is illustrated by spectra showing the frequency variation of certain coefficients of the spherical harmonic Y. We obtained the spectra by saving the spherical harmonic coefficients averaged with respect to pressure, every 10 (model) days, and applying a fast Fourier transform to the data for the last 96 years of the run. We excluded the first few years of the run to eliminate any initial transient effects. Figure 2*a* shows the spectrum of the Y_1^0 temperature coefficient. This is a measure of the inter-hemispheric

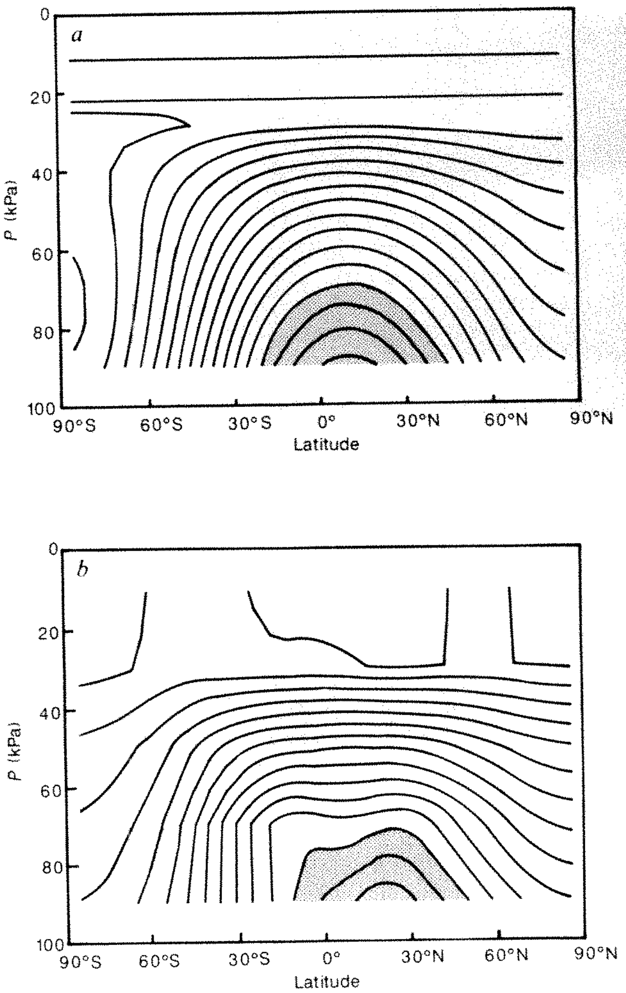

FIG. 1 Latitude–pressure cross-sections showing the zonal mean temperature distribution in *a*, the radiative–convective equilibrium state at the solstices and *b*, the model-generated solstice climatology. Contour interval 5 K, values in excess of an arbitrary reference temperature shaded.

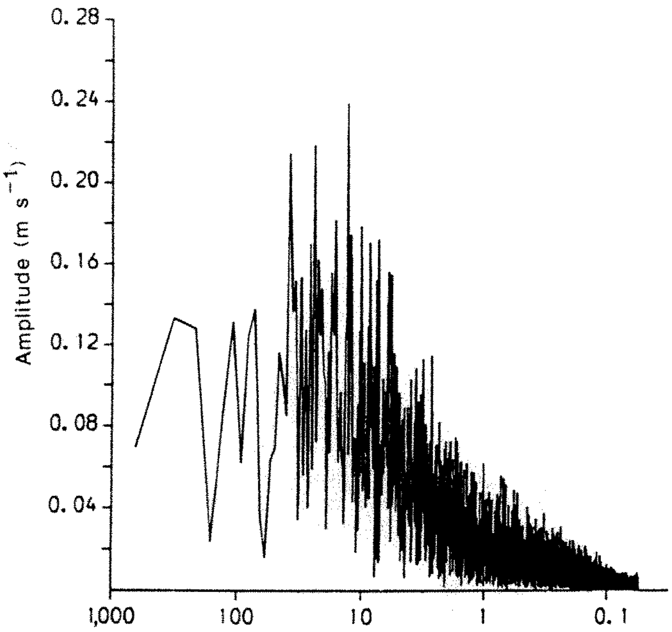

FIG. 4 As Fig. 2*b*, but for the 648-yr constant solstice calculation.

We fitted an autoregressive model to the time series of Y_1^0 coefficients. A 'red-noise' spectrum, with maximum variability at zero frequency gave the best fit. Standard statistical tests demonstrate the sharp peaks close to the period of the solar cycle are not *a posteriori* statistically different from the red-noise background. Rather, the spectra seem to be characterized by broad peaks with decadal periods. We extended the constant-solstice run to 648 years to test this suggestion. The spectrum of the Y_1^0 vorticity coefficient is shown in Fig. 4. A number of rather sharp peaks are seen in the range 10–40 years; the smoothed spectrum peaks in this range, and falls off towards both lower and higher frequencies. The maximum variability is clearly located within this range of periods.

The internal variability of the zonal mean part of the atmospheric circulation is large, on timescales as long as are currently available in accurate global data sets or in runs of global-circulation models. There is no reason to appeal to forcings external to the atmosphere (for example, solar forcing) to account for ultra-low-frequency variability. Models such as ours, with sophisticated atmospheric dynamics but simplified representation of other processes, are a benchmark against which to measure the ultra-low-frequency variability of more complex climate models. ☐

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1. Labitzke, K. & van Loon, H. *J. atmos. terr. Phys.* **50,** 197–206 (1988).
2. van Loon, H. & Labitzke, K. *J. Clim.* **1,** 905–920 (1988).
3. Geller, M. A. *Nature* **332,** 584–585 (1988).
4. Hide, R. & Mason, P. J. *Adv. Phys.* **24,** 47–100 (1975).
5. Shutts, G. J. *Q. Jl R. met. Soc.* **109,** 737–761 (1983).
6. James, I. N. & Gray, L. J. *Q. Jl R. met. Soc.* **112,** 1231–1250 (1986).
7. Wallace, J. M. & Blackmon, M. L. in *Large Scale Dynamical Processes in the Atmosphere* (eds Hoskins, B. J. & Pearce, R. P.) 55–94 (Academic, London, 1983).
8. Chatfield, C. *The Analysis of Time Series: An Introduction* 3rd edn, 142–143 (Chapman and Hall, London, 1984).

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Critical currents near 10^6 A cm^{-2} at 77 K in neutron-irradiated single-crystal $YBa_2Cu_3O_7$

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THE defects in as-grown bulk $YBa_2Cu_3O_7$ have been shown to act as weak flux pinning sites, leading to a reversible magnetization[1], magnetization decay[2] and non-zero resistivity[3] in moderate magnetic fields at temperatures near the transition temperature, T_c. These effects limit the technological utility of the as-grown material[3]. This is not entirely surprising, as both single crystals and polycrystalline materials are grown under conditions expected to minimize the defect density, but the weak pinning in these materials has nevertheless raised the issue of a possible intrinsic limitation to the critical current density. Here we report the attainment of a critical current density, J_c, of $\sim 6\times10^5$ A cm^{-2} at 77 K and 9 kOe, in a single crystal of $YBa_2Cu_3O_7$ irradiated with fast neutrons to introduce defects. (In both irradiated and unirradiated crystals, the critical current density was inferred by measuring the magnetic moment resulting from an induced persistent current.) This hundredfold enhancement of J_c relative to unirradiated crystals demonstrates that artificially induced defects can act as strong flux pinning sites, and analysis of the defects should increase our understanding of flux pinning in high-T_c superconductors.

Thin films, in which we expect both different defects and a far higher defect density, can sustain supercurrents of $>4\times 10^5$ A cm^{-2} at 77 K and 20 kOe, with low dissipation levels[4] (measured by magnetization). Until now, such high values have not been reported in bulk $YBa_2Cu_3O_7$. Various methods of artificially increasing the defect density have been tried, including ion irradiation[5,6] and cation substitution, but the critical current density at 77 K has not been significantly enhanced.

Fast neutron irradiation[7–11] has also been used, with results ranging from negative to promising. Küpfer *et al.*[11], for example, report a twentyfold increase in the intragranular J_c of polycrystalline $YBa_2Cu_3O_7$ at 40 K and 15 kOe. As these ceramics are prepared using a high-temperature sintering step, the intragranular defects, and hence J_c, are similar to those of single crystals[12]. Wisniewski *et al.*[10], found a twentyfold increase in the magnetization of ceramic at 77 K after neutron irradiation, but the intragranular J_c cannot be inferred from the reported data. Fleischer *et al.*[13] found a similar twentyfold increase after irradiation of uranium-doped $YBa_2Cu_3O_7$ with thermal neutrons, and were able to infer critical currents of $\sim 10^5$ A cm^{-2} at 77 K. Previous work on single crystals[8] has suggested at best a twofold increase in J_c, even though a procedure similar to ours was used.

Our single crystals were prepared as described previously[14]. Extensive measurements on these crystals have convinced us that they are uniform, homogeneous, reproducible and generally of high quality. They were encapsulated in silica tubes and irradiated with fast ($E > 1.0$ MeV) neutrons at the reactor facility of the University of Virginia, a facility which offers a high degree of shielding from slow neutrons (Cd ratio = 1.0). The neutron fluence was 7.9×10^{16} cm^{-2}.

Figure 1 shows the magnetization of an irradiated crystal during cooling in a 30-Oe applied field ($H \parallel c$ axis). The transition temperature is unchanged from the unirradiated value (89 K) and the sample shows no low-temperature anomalies which might indicate extensive second-phase regions. (The low level of flux expulsion results from the thin-plate geometry.) Zero-field-cooled measurements show that at low temperatures the sample exhibits essentially complete diamagnetic shielding. These results are consistent with previous studies[8,9]. Magnetization loops ($H \parallel c$ axis), shown in Fig. 2, were obtained with a vibrating-sample magnetometer. Owing to the low critical current of the unirradiated sample, an ensemble of nominally identical crystals was measured to increase the signal-to-noise ratio. The limited number of samples has allowed us to measure only one irradiated crystal so far, so we cannot report on the reproducibility of the experiment. The magnetization of the irradiated crystal (at $H=0$) did not decay measurably over a time interval of ~30 s; this behaviour will be the subject of a further study. A crude estimate of the self-inductance of the sample is ~1 nH; thus, the inferred resistivity is in the range $\rho < 10^{-16}\ \Omega$ cm, assuming that the decay is dominated by a simple L/R time constant (where L is inductance and R resistance). The dimensions of the sample ($0.06 \times 0.08 \times 0.003$ cm) preclude a satisfactory estimate of critical-current anisotropy[15], as the broad face is in the a–b plane.

Transport measurements of J_c are both insensitive (to measure $10^{-16}\ \Omega$ cm at 10^6 A cm^{-2} with conventional equipment would require a crystal ~1 m long) and necessitate the application of a large current (for a typical cross-section of 0.06×0.003 cm, a current of 240 A would be required). But we can infer the critical current in the plane of the film ($J \parallel a, b$ axes) unambiguously using the critical-state model and a Bean-like interpretation. In this case, $J_c = 20\,\Delta M[f(1-f/3g)]^{-1}$ (ref. 15) where ΔM is the difference between the upper and lower magnetization branches at a given field, and f and g ($f<g$) are the face dimensions of the rectangular sample. Here, as throughout the paper, we use the practical units gauss, cm and amperes. At 9 kOe we measure $\Delta M^{\mathrm{irrad}} = 1{,}200$ G, so $J_c^{\mathrm{irrad}}(77\ \mathrm{K}, 9\ \mathrm{kOe}) = 6.2 \times 10^5$ A cm^{-2}. A similar analysis of the magnetization of the unirradiated sample yields $J_c^{\mathrm{unirrad}}(77\ \mathrm{K}, 9\ \mathrm{kOe}) = 6.5 \times 10^3$ A cm^{-2}. Measurements on an unirradiated sample broken from the same original crystal as the irradiated sample were noisy but consistent with the latter value. In these analyses, we assume that the currents flow through the entire sample, that is, that the sample is not granular. To check this, we note that the shielding field H^* required to reverse the direction of the circulating current is given by $H^* \approx 2\pi t J_c/5$, a value which depends only on the thickness, t, of the sample, and which has been empirically verified on many of our single crystals. The shielding field can be seen to be ~3 kOe, so $J_c \approx 8 \times 10^5$ A cm^{-2}. Unless the induced granularity coincidentally preserves the aspect ratio of the sample, this indicates that the induced currents flow throughout the sample. Note that if the sample were granular, the inferred intragranular critical current would be larger than 6×10^5 A cm^{-2} (inversely proportional to the characteristic length of a grain).

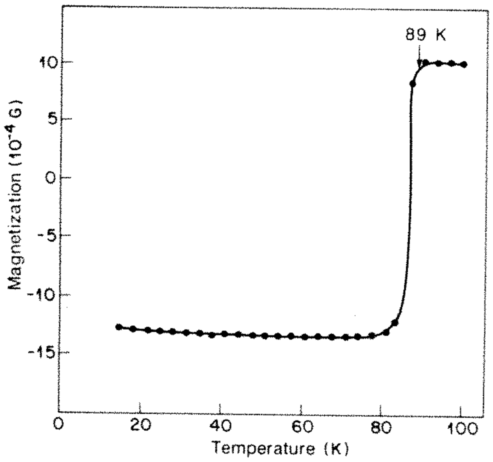

FIG. 1 Temperature dependence of the magnetization for the irradiated sample on cooling in a 30-Oe magnetic field. The absence of structure indicates a relatively homogeneous sample. The offset in magnetization above T_c is due to instrumental background.

We attribute the dramatic increase in J_c on irradiation to the introduction of pinning centres which inhibit flux motion. It is possible that the density of pinning centres has been increased, or that the pinning energy of each centre has increased, or both. Alternatively, the nature of the pinning may have changed, for example, by altering the electronic anisotropy. Note that the anomalous increase in J_c at low fields (>5 kOe) has been eliminated in the irradiated sample; a similar effect was noted previously in measurements[11] at 40 K, but the interpretation remains controversial. In any case, we emphasize that this magnetic measurement probes the sample with extreme sensitivity to dissipation, and that an effective criterion of $10^{-16}\ \Omega$ cm is sufficiently stringent for almost all technological applications.

The preceding discussion shows that flux creep is certainly not an intrinsic limitation on the use of bulk high-T_c superconductors. Radiation-induced defects are not expected to improve intergranular superconducting connections, as shown by studies on thin films[16], but in bulk materials not dominated by weak-link intergranular connections (for example, materials formed by melt-textured growth[17-19]), the artificially induced defects should dramatically enhance the critical current density at 77 K.

The structure of the defects introduced by neutron irradiation has not yet been determined. The defects apparently have a different effect than those produced by heavy-ion irradiation, which have so far given at most a twofold increase[6] in J_c at 77 K (in thin films). Irradiation by heavy ions creates a high density of defects strongly correlated along the tracks of individual ions. Because neutron collision cross-sections are small, neutron irradiation generates defects much more homogeneously

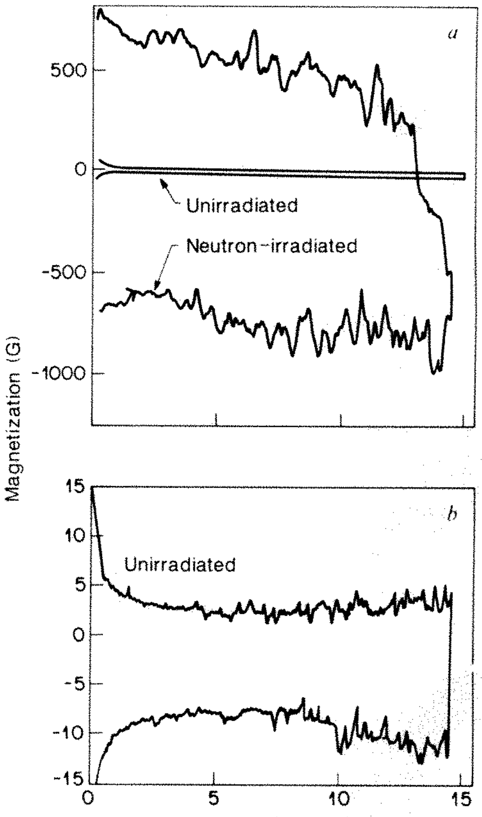

FIG. 2 *a*, Magnetization hysteresis loops of the unirradiated and irradiated sample. The large magnetizations observed in the irradiated sample imply a large circulating supercurrent. *b*, The hysteresis loop of the unirradiated sample on a more sensitive scale. The unirradiated sample is an ensemble of individual crystals, to increase the signal-to-noise ratio.

throughout the volume of our single-crystal samples, and with much lower local defect density. These distinctions may be important, but we also note that nominally identical neutron irradiation procedures have resulted in effects on critical current ranging from moderate enhancement to degradation. ☐

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1. Müller, K. A., Takashige, M. & Bednorz, J. G. *Phys. Rev. Lett.* **58**, 1143–1145 (1987).
2. Yeshurun, Y. & Malozemoff, A. P. *Phys. Rev. Lett.* **60**, 2202–2205 (1988).
3. Palstra, T. T. M., Batlogg, B., van Dover, R. B., Schneemeyer, L. F. & Waszczak, J. V. *Appl. Phys. Lett.* **54**, 763–765 (1989).
4. Watanabe, K. *et al. Appl. Phys. Lett.* **54**, 575–577 (1989).
5. Valles, J. M. *et al. Phys. Rev.* B**39**, 11599–11602 (1989).
6. Roas, B., Hensel, B., Saemann-Ischenko, G. & Schultz, L. *Appl. Phys. Lett.* **54**, 1051–1053 (1989).
7. Küpfer, H. *et al. Z. Phys.* **69**, 167–171 (1987).
8. Umezawa, A. *et al. Phys. Rev.* B**36**, 7151–7154 (1987).
9. Cost, J. R., Willis, J. O., Thompson, J. D. & Peterson, D. E. *Phys. Rev.* B**37**, 1563–1568 (1988).
10. Wisniewski, A. *et al. Solid St. Commun.* **65**, 577–580 (1988).
11. Küpfer, H. *et al.* in *The Science of Superconductivity and New Materials* (ed. Nakajima, S.) 172–182 (World Scientific, Singapore, 1989).
12. Jin, S. *et al. Appl. Phys. Lett.* **54**, 584–586 (1989).
13. Fleischer, R. L., Hart, H. R., Lay, K. W. & Luborsky, F. E. *Phys. Rev.* B**40**, 2163–2169 (1989).
14. Schneemeyer, L. F. *et al. Nature* **328**, 601–603 (1987).
15. Gyorgy, E. M., van Dover, R. B., Jackson, K. A., Schneemeyer, L. F. & Waszczak, J. V. *Appl. Phys. Lett.* **55**, 283–285 (1989).
16. White, A. E. *et al. Appl. Phys. Lett.* **53**, 1010–1012 (1988).
17. Jin, S. *et al. Appl. Phys. Lett.* **52**, 2074–2076 (1988).
18. Murakami, M., Morita, M., Doi, K. & Miyamoto, M. *Jap. J. appl. Phys.* **28**, 1189–1194 (1989).
19. Salama, K., Selvamanickam, V., Gao, L. & Sun, K. *Appl. Phys. Lett.* **54**, 2352–2354 (1989).

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Water-level fluctuations of Lake Tanganyika in phase with oceanic changes during the last glaciation and deglaciation

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THERE has been considerable controversy about the magnitude of fluctuations of the levels of Lake Tanganyika, the Earth's second deepest lake (1,470 m), following the discovery of submerged valleys extending down to 550 m below present lake levels[1]. These fluctuations register changes in the precipitation/(evaporation + evapotranspiration) ratio in a large equatorial–tropical area of catchment, south of the Equator. Here we report new palaeohydrological data, back to 40 kyr BP, from carbon dating of the total organic matter in two diatomaceous cores. The results constrain the vertical lake level fluctuations more than has hitherto been possible[2–5] and show that the fluctuations from 26 kyr BP are correlated with changes in global sea level and ice volume. Surprisingly fluctuations seem to be in phase with those of the African lakes north of the Equator, which are clearly linked to the Milankovitch mechanisms.

Lake Tanganyika (3°30′–9° S, 31°20′ E), a meromictic lake[6,7], contains ~18,800 km^3 of water[8]. The precipitation on the lake surface and surface runoff represents about 63% and 37% of the water input, respectively; evaporation (94%)[3,9] is the major loss of water. Surface outflow by the Lukula river controls the maximum (and present) lake level.

Core MPU XII (Figs 1 and 2), 1,013 cm in length, was collected in the southern basin where the lake is 422 m deep, and has been dated to 25,600 BP at its base[4]. Core MPU III (Figs 1 and 3), 1,123 cm in length, was taken at the depth of 130 m on the steep slope that separates the Cameron Bay from the southern basin. The sediments consist mainly of laminated or homogeneous diatomite.

Detailed results of quantitative diatom analyses and taxonomical discussion will be presented elsewhere. Here we focus on the water level indicators (Figs 2 and 3). Diatom biozones, based on the dominant taxa, appear in the cores (Fig. 4). We correlate them with diatom biozones previously established in core T2[2,3] which was collected close to core MPU XII (Figs 1 and 4). We have also deduced the ages of the Holocene events from core SD2 which was taken from the northern basin (Fig. 4).

We measured the stable isotope (^{13}C) contents of total organic matter (TOM) from samples of the core MPU III (Fig. 3) to evaluate the relative proportion of lacustrine and terrestrial organic matter[10]. The ^{13}C contents are close to −25‰ and the ^{14}C activity values (from accelerator mass spectrometry, AMS) thus did not require normalization[11].

Core MPU XII shows nine diatom biozones distributed in three major stages (Figs 2 and 4). During stage 1 (>25,600–21,700 yr BP), the lake was shallower than today, as indicated by the amount of *Cyclotella ocellata*, a facultative planktonic species commonly regarded as littoral[12]. This species predominates in biozones IA (>25,600–~24,500 yr BP) and IC (~22,500–21,700 yr BP). The scarcity of periphytic forms (attached on a submerged substrate) is, however, difficult to reconcile with very shallow environments, or with a massive transport of diatoms from the marginal zone. Modern analogues are not available for assemblages rich in *Cyclotella ocellata* in recent African lakes[13] although this species was common in the plankton of Ethiopian and Afar lakes during the late Pleistocene[14], such as Lake Abhé which was then 100–160 m deep[15]. Such a moderate depth at the MPU XII site would be compatible with both the low percentages of periphytic forms and with the absence of lamination which suggests that the top of the anoxic deep-water mass, presently lying at a depth of 100–150 m (ref. 6), was below the core site during the sedimentation of biozones IA and IC. In contrast, the laminated facies in biozone IB indicates a permanent water stratification. The relatively high proportion of planktonic forms and the permanency of *C. ocellata* are further indications of a moderate rise in water level centred around 24,000 yr BP.

Stage II (21,700–12,700 yr BP) corresponds to generally very shallow conditions. Periphytic diatoms (such as *Rhopalodia* spp.) became abundant. They are associated with sponge spicules and phytoliths which also reflect shallow environments. Few laminations are observed. The minimum water depth is recorded in biozone IIB by a peak of *Gomphonema clevei*, typically periphytic in modern East Africa[13]. The presence of intact frustules (connected valves) is a further indication that this species was growing *in situ* at the MPU XII site, which thus was located in the photic zone. This reveals a drop in water

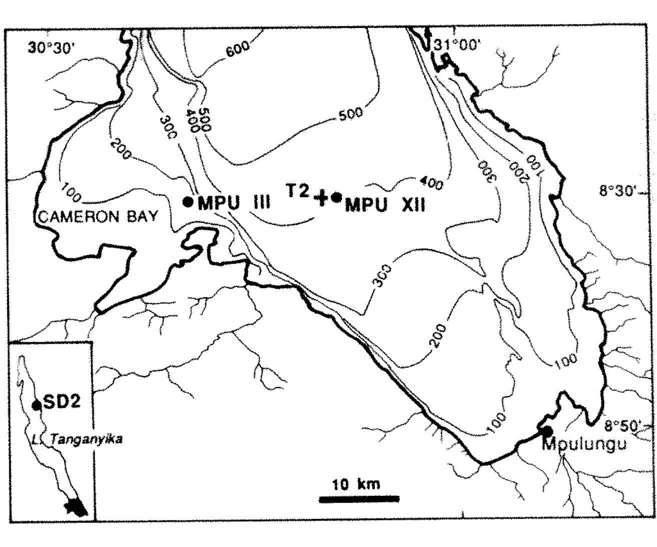

FIG. 1 Core location in Lake Tanganyika.

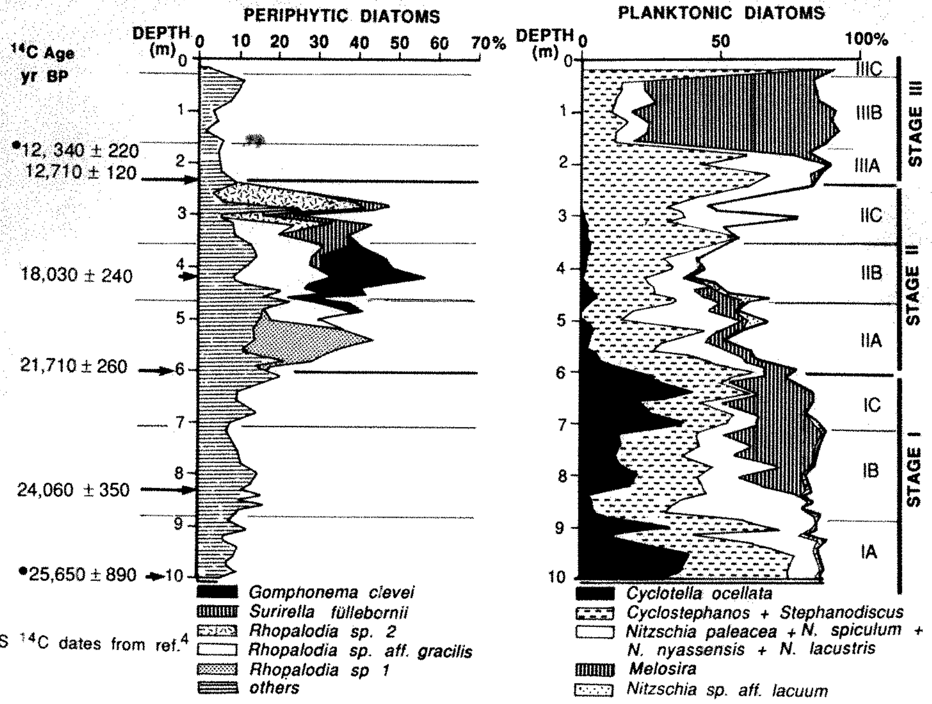

FIG. 2 Distribution of periphytic and planktonic diatoms along core MPU XII.

level of at least 350 m, more probably of about 400 m; this corresponds to a water volume that is −40 to −60% of the present value. The lake area/drainage area ratio was only 0.090–0.070 at that time compared to a present value of 0.137. The *Gomphonema* peak is dated at 18,030 ± 240 yr BP. This date differs significantly from that previously inferred from the ^{14}C-dated secular palaeomagnetic variations obtained in a Cameroon lake and extrapolated to Lake Tanganyika[4]. Whatever the causes for this discrepancy (for example, limited resolution of conventional ^{14}C ages from the Cameroon materials or delayed magnetic reorientation of fine particles in the highly porous sediments from Lake Tanganyika), such extrapolations seem to be unwarranted.

A generally positive lake water balance was established during Stage IIC (~16,000- > 12,700 yr BP), and the planktonic flora indicates that deep conditions were definitely restored to the lake shortly before 12,700 yr BP. This event was possibly preceeded by a first-positive oscillation around 14,500 yr BP, as suggested by a narrow peak of planktonic forms observed at a depth of 300 cm (Fig. 2). Deep-water conditions were maintained during Stage III, from 12,700 yr BP onwards. Perennial stratification occurred from 12,700- ~10,600 yr BP (base of biozone IIIA; Fig. 4) and from ~5000 yr BP, whereas biozone IIIB indicates deep mixing which may be due to upwelling events[5].

In core MPU III (Fig. 3), detritus deposits and disturbances are observed in the intervals 981–935 cm and 880–780 cm, and we therefore do not consider them in our palaeohydrological reconstructions. A discontinuity is suggested by the ^{14}C dates between ~30,000 and >12,300 yr BP, which may explain the absence of stages I and IIA–B.

Core MPU III confirms that the major regression ends around 13,000 yr BP. We assign the core section 780–760 cm to biozone IIC, and deep-water conditions were established before 12,300 yr BP (biozone IIIA) and maintained to the present. From >12,300–10,600 BP, a progressive decrease in the ^{13}C values (Fig. 3) may reflect the establishment of an arboreal vegetal cover on the catchment area[4], indicating increasing temperature and humidity[16]. The marked decrease at ~10,600 BP indicates

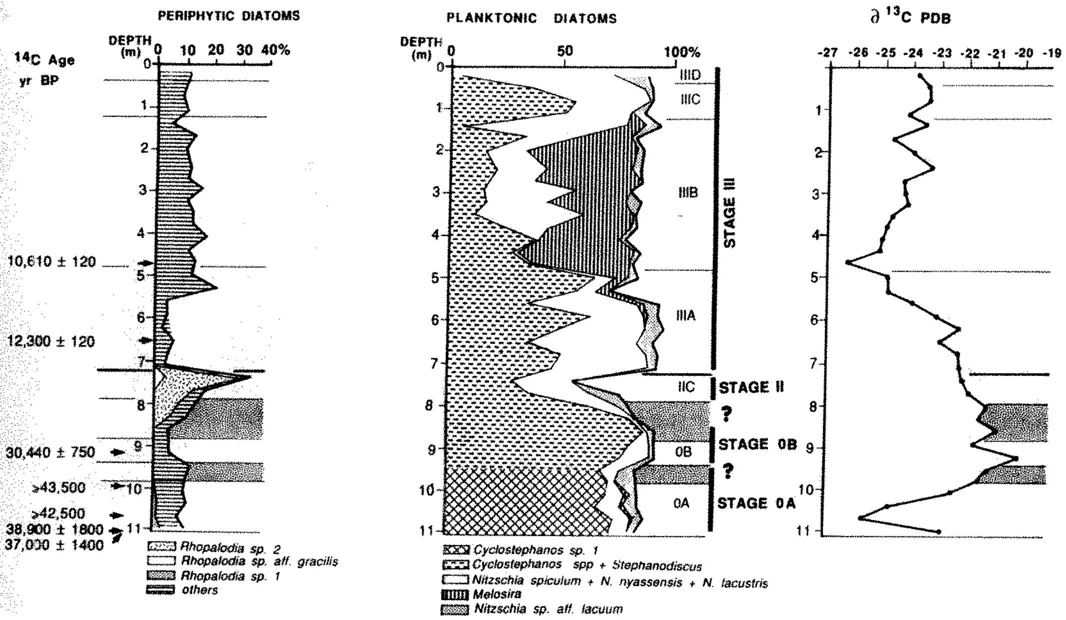

FIG. 3 Distribution of periphytic and planktonic diatoms, and ^{13}C content of total organic matter along core MPU III.

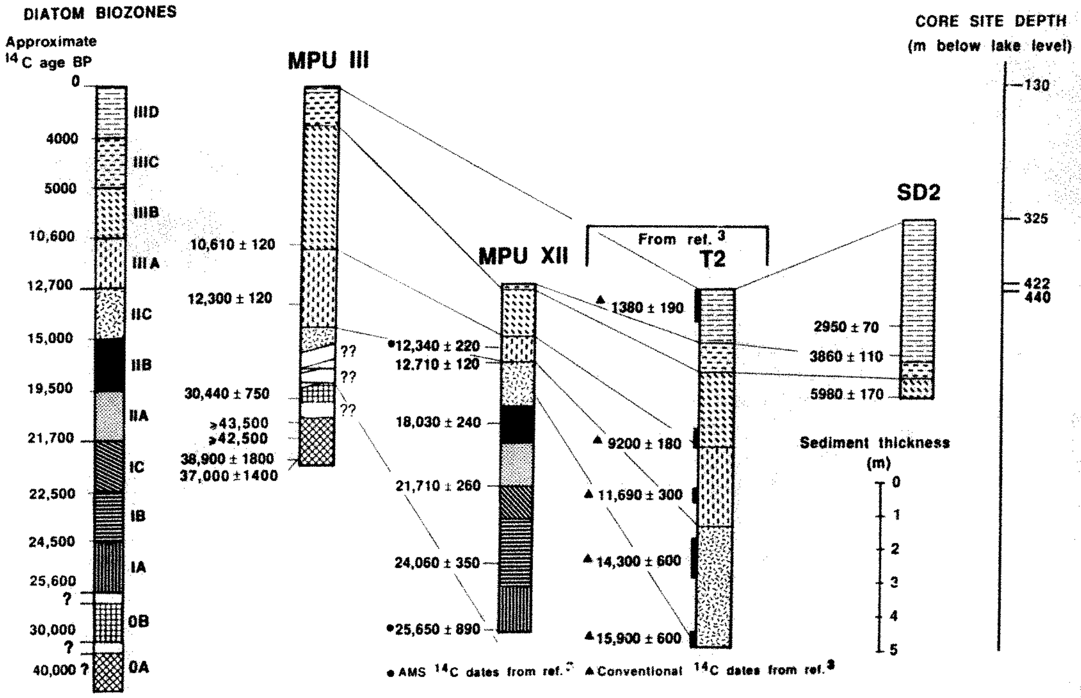

FIG. 4 Tentative correlations between four cores from Lake Tanganyika using diatom biozones. Core T2 from ref. 3. Diatom biozones, abundant taxa: 0 A, *Cyclostephanos* sp. 1; 0 B, *Cyclostephanos damasii*, needle-like *Nitzschia* (*N. spiculum*, *N. nyassensis*, *N. lacustris*; IA: *Cyclotella ocellata*, needle-like *Nitzschia*; IB: *Cyclostephanos* spp. +*Stephanodiscus* spp. needle-like *Nitzschia*, *Cyclotella ocellata*; IC: *Cyclotella ocellata*, *Melosira* spp.; IIA: *Rhopalodia* sp. 1., *Cyclostephanos* spp. +*Stephanodiscus* spp.; IIB: *Gomphonema clevei*, *Rhopalodia* sp. *aff. gracilis*; IIC: *Cyclostephanos* spp. +*Stephanodiscus*, *Surrirella fullebornii*, *Rhopalodia* spp., 2; IIIA: *Cyclostephanos* spp. +*Stephanodiscus* spp., needle-like *Nitzschia*; IIIB: *Melosira* spp.; IIIC: *Cyclostephanos damasii*; IIID: needle-like *Nitzschia*. (needle-like is the terminology used in ref. 3).

the maximum input of organic matter of terrestrial origin and an abrupt increase in rainfall and related runoff which coincides with the limit between biozone IIIA and IIIB.

Below 920 cm, the diatom flora reveals the occurrence of two Late Pleistocene stages of high water level (Fig. 3). The youngest, stage OB, is dated to 30,440 ± 750 yr BP. Facies and diatom flora resemble that of biozone IIIA. The ^{13}C content reflects an almost exclusive aquatic origin of the organic matter. The oldest, stage OA, is characterized by a planktonic diatom assemblage unknown in core MPU XII. Its age falls at or close to the limit of the ^{14}C method (Fig. 3). The slight discrepancies observed between the three measured ages may be due to the presence of detrital organic matter, because the low ^{13}C values indicate an important input of terrestrial material. Such a supply is indistinguishable in the core stratigraphy. An alternative explanation may thus be found in a slight contamination of the 1,108-cm sample which repeatedly provided a still measurable ^{14}C age (Fig. 3).

This survey shows that lake level fluctuations from 26,000 yr BP correlate with changes in global sea level and ice volume[17]: the lake level is intermediate from 26,000 to 21,700 yr BP, is low from 21,700 to 13,000 yr BP with a minimum at 18,000 yr BP and is high from 13,000 yr BP onwards. The reestablishment of a positive water balance occurred during the first step of the deglaciation, but the refilling of the lake is not linear. The abruptness of the major hydrological events recorded at ~13,000 yr BP and ~10,600 yr BP makes the fluctuations of the lake water balance synchronous with the deglaciation steps recorded in the Atlantic ocean[18,19]. Lake Tanganyika fluctuations seem to be in phase with those of the African lakes north of the Equator[20] which are clearly linked to the Milankovitch mechanisms[21]. From the general circulation models we might have expected opposite phases as a result of different insolation values from the astronomical theory of palaeoclimates[22]. Conditions wetter than the present ones, which are predicted by a simulation[23] at 18,000 yr BP in East Africa and attributed to warmer surface temperature of the equatorial Indian Ocean, are not confirmed. More simply, the water balance of Lake Tanganyika reflects the hydrological ocean variations that are due to glaciation/deglaciation processes and the related greater availability of global atmospheric moisture during warmer global climatic phases. ☐

Note added in proof: We have become aware of a related paper[24] which considers the possibility of a time lag of some centuries in the accumulation of organic matter. This hypothesis is based on the isotope balance of C_4/C_3 plants as an index of *in situ* (*Cyperaceae*) versus reworked (trees) organic matter. However, the planktonic contribution, with ^{13}C content lower than for *Cyperaceae*, is neglected, and thus the time-lag estimate is pessimistic.

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1. Capart, A. *Exploration hydrobiologique du Lac Tanganyika (1946-47)*, vol. 2, 1-16 (Inst. R. Sci. Nat. Belg., Bruxelles, 1949).
2. Livingstone, D. A. *Limnol. Oceanogr.* **10**, 607-610 (1965).
3. Haberyan, K. A. & Hecky, R. E. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **61**, 169-197 (1987).
4. Tiercelin, J. J. *et al. C. R. hebd. Séanc. Acad. Sci., Paris* **307**, 1375-1382 (1988).
5. Scholz, C. A. & Rosendahl, C. A. *Science* **240**, 1645-1648 (1988).
6. Degens, E. T., Van Herzen, R. P. & Wong, H. K. *Naturwissenschaften* **58**, 229-251 (1971).
7. Stoffers, P. & Hecky, R. E. in *Int. Ass. Sedimentol., Spec. pub. 2*, 43-55 (1978).
8. Hecky, R. E. & Degens, E. T. *Woods Hole Oceanographic Institution, Tech. Rep.* 73-78 (1978).
9. Beadle, L. C. *The Inland Waters of Tropical Africa* (Longman, London, 1981).
10. Deines, P. in *Handbook of Environmental Isotope Geochemistry* Vol. 1 (eds Fritz, P. & Fontes, J. Ch.) 329-406 (Elsevier, Amsterdam, 1980).
11. Stuiver, M. & Polach, H. A. *Radiocarbon* **19**, 355-363 (1977).
12. Hustedt, F. in *Das Phytoplankton des Süsswassers* Vol. 16 (ed. Thiennemann, A.) (Schweizerbart, Stuttgart, 1942).
13. Gasse, F. in *Bibliotheca Diatomologica* Vol. 11 (Cramer, Stuttgart, 1986).
14. Gasse, F. & Street, F. A. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **24**, 279-325 (1978).
15. Gasse, F. *Nature* **2**, 42-45 (1977).
16. Vincens, A. *Palaeobotany and Palynology* (Elsevier, Amsterdam, in the press).
17. Labeyrie, L. D., Duplessy, J. C. & Blanc, P. L. *Nature* **367**, 477-482 (1987).
18. Bard, E. *et al.* **328**, 791-794 (1987).
19. Berger, W. H., Killingley, J. S. & Vincent, E. *Nature* **314**, 156-158 (1985).
20. Street-Perrott, F. A. & Roberts, N. in *Variations in the Global Water Budget* (eds Street-Perrott, F. A. *et al.*) 331-445 (Reidel, Dordrecht, 1983).
21. Kutzbach, J. E. & Street-Perrott, F. A. *Nature* **317**, 2130-2134 (1985).
22. Berger, A. *J. atmos. Sci.* **35**, 2362-2367 (1978).
23. COHMAP Members. *Science*, **241**, 1043-1052 (1988).
24. Hillaire-Marcel, C. *et al. Quat. Sci. Rev.* **8**, 206-211 (1989).

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Glacial–interglacial climatically forced $\delta^{13}C$ variations in sedimentary organic matter

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SEDIMENTARY organic geochemical properties have long been used to characterize sedimentary organic matter sources[1–8], but the characterizations are often not unique. There are at least four plausible mechanisms to account for the large (~3–6‰) glacial-to-interglacial climatic variations in the sedimentary organic-carbon isotopic composition ($\delta^{13}C_{org}$) in the northern Gulf of Mexico. They are: temperature-dependent C isotopic fractionation by phytoplankton, mixing of C_3-photosynthetic terrigenous and marine organic matter, mixing of C_3- and C_4-photosynthetic organic matter and diagenetic alteration of the originally sedimented material. In a 208.7-m Deep Sea Drilling Project hydraulic-piston core (DSDP 619) from the Pigmy Basin in the northern Gulf of Mexico, we find that paired analyses of $\delta^{13}C_{org}$ and N/C are consistent with the hypothesis that the sedimentary organic carbon in the Pigmy Basin is a glacial-interglacial climatically determined mixture of C_3-photosynthetic terrigenous and marine organic matter[9], confirming the model of Sackett[5].

The Pigmy Basin is a late Quaternary blocked-canyon intraslope basin the northern Gulf of Mexico[10] (Fig. 1). The basin has normally oxic and saline bottom waters[11]. In interglacial times (isotope (sub)stages 1 and 5a,b) sedimentation rates ranged from 60 to 85 cm kyr^{-1}, whereas during glacial times (isotope stages 2 and 4) sedimentation rates varied from 230 to 250 cm kyr^{-1} as determined from the DSDP Hole 619 hydraulic piston core[9,12]. The occurrence of higher sedimentation rates in glacial stages was presumably due to increased erosion of continental material during falling sea levels and low sea-level stands[13].

We present the sedimentary organic-carbon isotopic composition ($\delta^{13}C_{org}$) of 55 samples from the Pigmy Basin as a time series (Fig. 2*a*). The carbon isotope results are given in the δ-notation relative to the Chicago PDB standard, $\delta^{13}C_{org} = (R_S/R_R - 1) \times 1{,}000$ where R_S and R_R are the $^{13}C/^{12}C$ ratios of sample and reference CO_2 gases[4]. The interglacial oxygen-isotope stages 1 and 5a,b are characterized by relatively positive average ($\pm 1\sigma$) $\delta^{13}C_{org}$ values (stage 1: -24.2 ± 1.2‰, $n = 9$; substages 5a,b: -23.0 ± 0.8‰, $n = 7$), whereas stages 2 and 3 have comparatively negative average values (stage 2: -25.6 ± 0.5‰, $n = 13$; stage 3: -25.2 ± 1.3‰, $n = 18$). Isotope stage 4 has two relatively negative values (average -25.3‰) in the middle of the stage with relatively positive values near the stage 4 boundaries. (3/4 boundary: -22.8‰; 4/5 boundary -21.9‰). To our knowledge this time series is the highest-resolution record of $\delta^{13}C_{org}$ in the Gulf of Mexico spanning from the present interglacial isotope stage 1 to the previous interglacial isotope substage 5b (~96 kyr BP).

Sackett observed a 4.8‰ increase in the carbon isotopic composition of sedimentary organic matter over the last ~20 kyr on the Sigsbee Knolls (core V3-127) in the northern Gulf of Mexico[5]. He attributed the temporal $\delta^{13}C_{org}$ increase to mixing of terrigenous and marine organic carbon sources by analogy with the apparent spatial mixing of terrigenous and marine organic carbon in Recent eastern Gulf Coast surface sediments. The temporal variation of $\delta^{13}C_{org}$ is consistent with a glacial-to-interglacial climatically forced mixing of terrigenous and marine organic carbon[5] and the effects of the ~100-kyr cycle of climatic change[14].

Rogers and Koons[6] examined the $\delta^{13}C_{org}$ records of 24 gravity and piston cores and four 330-m drill cores in the northern Gulf of Mexico, with the longest record extending into the upper Pliocene (~2 Myr). They found that warm stages (as defined by planktonic foraminiferal assemblages) were characterized by $\delta^{13}C_{org}$ values of -20 to -22‰ (relative to PDB), whereas cold stages had $\delta^{13}C_{org}$ values of -24 to -26‰. They eliminated sedimentary diagenesis of organic carbon as a cause for $\delta^{13}C_{org}$ variations because over the time spanned (~2 Myr) there was no secular monotonic trend in $\delta^{13}C_{org}$ values. They concluded that the major cause of the $\delta^{13}C_{org}$ variations was the temperature-dependent fractionation of carbon isotopes by phytoplankton, and considered mixing of terrigenous and marine organic carbon an unlikely cause.

Given the sedimentary $\delta^{13}C_{org}$ variations in the northern Gulf of Mexico, Sackett and Rankin[7] showed that the glacial-to-interglacial sea surface temperature variation predicted from the temperature-induced fractionation of carbon isotopes (35 °C) would be implausibly large in light of the oxygen isotopic composition of contemporaneous planktonic foraminifera[15]. As a temperature change of only 6–10 °C from the last glacial to the present interglacial had been calculated earlier with the $\delta^{18}O$ of planktonic foraminifera in Caribbean surface waters[15], they favoured the variation of relative proportions of terrigenous and marine organic carbon inputs as the cause of the temporal $\delta^{13}C_{org}$ changes. Later, the applications of a foraminiferal-assemblage temperature-transfer method[16,17] and the foraminiferal oxygen-isotope temperature method using revised ice volume estimates[9,18] indicated only small (0.8–2 °C) late Quaternary temperature variations in the northern Gulf of Mexico. These results make temperature-dependent carbon

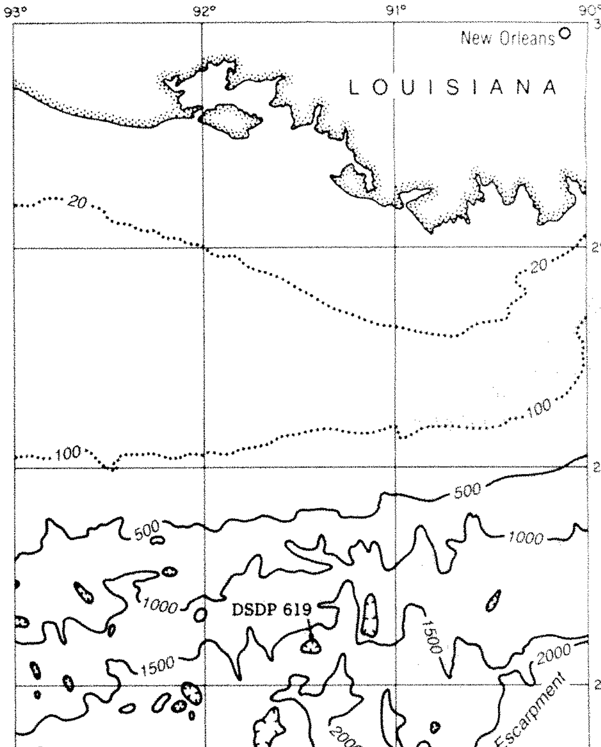

FIG. 1 Map showing cored site on Deep Sea Drilling Project Leg 96, including the Pigmy Basin (Site 619) at 27°11.61′ N, 91°24.54′ W.

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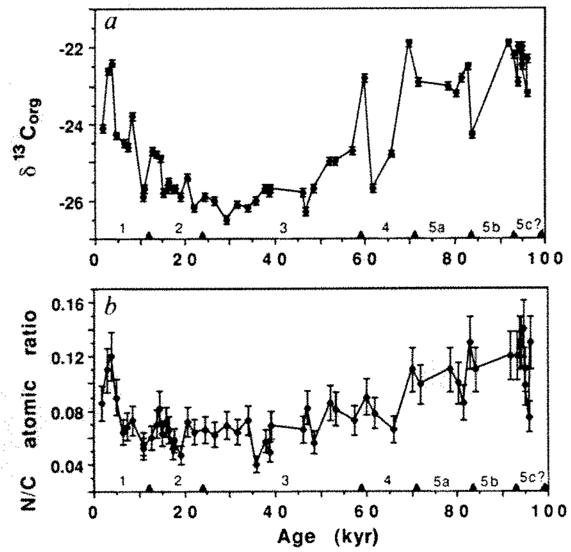


FIG. 2 *a*, Time series of the sedimentary organic-carbon isotopic composition ($\delta^{13}C_{org}$) of DSDP 619 in the Pigmy Basin, showing relatively positive (marine) values in interglacial stages and relatively negative (terrigenous) values in glacial stages. *b*, Time series of N/C atomic ratios in DSDP 619 shows the general interglacial predominance of marine organic matter (typically, N/C = 0.13–0.20) and the glacial predominance of terrigenous organic matter (typically, N/C = 0.01–0.05).

isotopic fractionation an unlikely explanation for the large $\delta^{13}C_{org}$ variations observed in the DSDP 619 core.

Neuman *et al.*[8] observed that the $\delta^{13}C_{org}$ values of "olive grey, Pleistocene turbidite muds" were −22 to −24‰ (relative to PDB) and that the $\delta^{13}C_{org}$ values for "orange and yellow, Holocene and interglacial foraminiferal oozes" were −20 to −16‰. Analysis of the $\delta^{13}C_{org}$ records in 48 piston cores in all the major deep-water provinces of the late Quaternary Gulf of Mexico led them to conclude that the temporal $\delta^{13}C_{org}$ variations were consistent with increased proportions of terrigenous organic-carbon deposition relative to marine organic-carbon deposition in glacial stages over broad expanses of the Gulf's sea floor.

The final alternative is that the sediments of the northern Gulf of Mexico contain a climatically determined mixture of C_3- and C_4-photosynthetic organic matter. Terrigenous C_3-photosynthetic organic matter could be eroded into the Gulf from terrigenous sources in glacial times. In interglacial times, C_4-organic matter could be contributed from C_4-photosynthetic coastal plants[19] or other ^{13}C-rich sea grasses[20] into the deep Gulf of Mexico. We consider this possibility below.

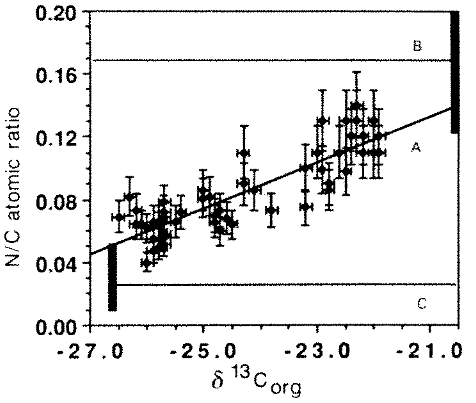
The N/C ratios of DSDP 619 sediments are presented as a time series (Fig. 2*b*). The interglacial (sub)stages 1 and 5a-b are characterized by relatively high N/C values (stage 1: 0.08 ± 0.02 ($\bar{X} \pm 1\sigma$), $n = 9$; substages 5a,b: 0.11 ± 0.02, $n = 7$). These N/C values approach those of planktonic organic matter[1] (0.13–0.20). The average N/C value of glacial stages 2-4 is 0.06 ± 0.02 which overlaps with the range of typical terrigenous plant and peat values of 0.01–0.05 (refs 2, 3). The N/C time series for the Pigmy Basin is consistent with the model of climatically forced glacial-to-interglacial terrigenous- and marine-organic-carbon mixing, reflecting the general ~100 kyr climatic cycle.

In Fig. 3 we plot 55 $\delta^{13}C_{org}$ values against their corresponding N/C values from the Pigmy Basin core. A linear least-squares regression of N/C on $\delta^{13}C_{org}$ shows that the measurements are well correlated ($r^2 = 0.74$, $P < 0.0001$) according to the statistical *F*-test for significance[21]. The correlation of N/C and $\delta^{13}C_{org}$ values for the sedimentary organic matter in the Pigmy Basin is consistent with a mixing of C_3-photosynthetic (Calvin–Benson Cycle of carbon fixation) terrigenous and marine organic matter sources. A linear regression line (labelled A) through the paired data extrapolates through typical terrigenous (0.01–0.05) and marine (0.13–0.20) N/C values at empirically determined $\delta^{13}C_{org}$ terrigenous (−26.6‰, relative to PDB) and marine (−20.6‰) end-member values[9].

These data support the climatically driven terrigenous- and marine-organic-carbon mixture model and are evidence against other hypotheses for organic-matter deposition in the late Quaternary Gulf of Mexico. If temperature-dependent fractionation of carbon isotopes by marine phytoplankton determined the Quaternary sedimentary $\delta^{13}C_{org}$ values, we would expect constant marine N/C values (0.13–0.20) to co-occur with variable $\delta^{13}C_{org}$ values (line B) contrary to observation. This observation is consistent with the assertion that the maximum Quaternary surface-water temperature variation in the Gulf of Mexico would cause only an ~1‰ difference in $\delta^{13}C_{org}$, if it had any effect at all[8]. If a climatically determined mixture of C_3- and C_4- terrigenous organic matter caused the temporal $\delta^{13}C_{org}$ variation, we would observe relatively constant terrigenous N/C values (0.01–0.05) across the range of $\delta^{13}C_{org}$ values (line C). This is also contrary to observation.

Sedimentary organic-carbon isotope results from coastal marshes to Recent deep oceanic environments, showed that there was no convincing evidence for significant diagenetic alteration of the sedimentary $\delta^{13}C_{org}$ records[22]. Although the possibility of diagenetic alteration of Quaternary $\delta^{13}C_{org}$ records has been discussed[6], we are unaware of results that support sedimentary diagenetic carbon isotopic alteration as the cause of large $\delta^{13}C_{org}$ variations (3–6‰) as those observed in the Quaternary Gulf of Mexico sediments[5-9]. Rogers and Koons[6] considered carbon isotopic fractionation during diagenesis to be an insignificant problem affecting the $\delta^{13}C_{org}$ record of 0-2-Myr-old sediments because they did not observe a secular trend in the $\delta^{13}C_{org}$ values. A later study of the $\delta^{13}C_{org}$ of the algal sapropelic sediments of the alternately aquatic, then marine Mangrove Lake in Bermuda, however, indicated an overall decrease in $\delta^{13}C_{org}$ of up to ~4‰ over the past 5 kyr (ref. 23). Therefore, $\delta^{13}C_{org}$ records should not be interpreted solely in

FIG. 3 This plot of N/C against $\delta^{13}C_{org}$ shows that the composition of sedimentary organic matter in the Pigmy Basin (DSDP 619) for the last ~100 kyr is consistent with mixing of terrigenous and marine C_3-photosynthetic (Calvin–Benson Cycle) organic matter. The linear least-squares regression line (A) is: $N/C = 0.015 \cdot (\delta^{13}C_{org}) + 0.44$ ($P < 0.0001$, $r^2 = 0.74$, $n = 55$). For comparison, typical N/C ratios of terrigenous (0.01–0.05) and marine (0.13–0.20) plants are given at $\delta^{13}C_{org}$ terrigenous (−26.6‰) and marine (−20.6‰) endmember values[9]. Constant marine N/C values with variable $\delta^{13}C_{org}$ values (line B) would support temperature-dependent carbon isotopic fractionation as the cause of the late Quaternary Gulf of Mexico $\delta^{13}C_{org}$ variations. Constant terrigenous N/C ratios for all $\delta^{13}C_{org}$ values would support the mixing of C_3- and C_4-terrigenous plant material as the cause of the $\delta^{13}C_{org}$ variations (line C). Neither of the latter two hypotheses (represented by lines B and C) are supported by these data.



terms of organic matter sources, particularly in organic-rich sapropels. In the Mangrove Lake, secularly increasing $\delta^{13}C_{org}$ values were the result of preferential degradation of selected organic-matter components[23]. This mechanism, however, cannot explain the glacial-interglacial cyclic co-variation in $\delta^{13}C_{org}$ and N/C values in the Pigmy Basin. Changes in C_3-photosynthetic terrigenous and marine organic matter sources are supported as the principal source (~74%) of the variation of the $\delta^{13}C_{org}$ and N/C records, whereas sedimentary carbon isotopic fractionation, temperature effects on phytoplanktonic carbon isotopic fractionation and mixing of C_3- and C_4-photosynthetic organic matter may account for some fraction of remaining variation (~26%). These organic-matter source changes are internally consistent with the inverse relations of selected marine (C_{37}–C_{39} alkenones) and terrigenous (C_{27}–C_{29} desmethyl sterols) organic biomarker compounds to $\delta^{13}C$ values also found in the Pigmy Basin sediment core (ref. 9; J.P.J. and R.B.G., manuscript in preparation). □

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1. Redfield, A. C., Ketchum, B. H. & Richards, F. A. in *The Sea*, Vol. 2 (ed. Hill, M. N.) 26-77 (Wiley, New York, 1962).
2. Waksman, S. A. *Soil Sci.* **36**, 125-147 (1933).
3. Brenner, S., Ikan, R., Agron, N. A. & Nissembaum, A. *Soil Sci.* **125**, 226-232 (1978).
4. Craig, H. *Geochim. cosmochim. Acta* **3**, 53-92 (1953).
5. Sackett, W. M. *Mar. Geol.* **2**, 173-185 (1964).
6. Rogers, M. A. & Koons, C. B. *Trans. Gulf Coast Ass. geol. Soc.* **19**, 529-534 (1969).
7. Sackett, W. M. & Rankin, J. G. *J. geophys. Res.* **75**, 4557-4560 (1970).
8. Neuman, J. W., Parker, P. L. & Behrens, E. W. *Geochim. cosmochim. Acta* **37**, 225-238 (1973).
9. Jasper, J. P. thesis, Mass. Inst. Tech./Woods Hole Oceanographic Inst., WHOI-88-28 (1988).
10. Bouma, A. H. *Mem. Am. Ass. Petrol. Geol.* **34**, 567-581 (1983).
11. Bouma, A. H., Stelting, C. E. & Leg 96 Sedimentologists *Init. Rep. DSDP* **96**, 563-576 (1986).
12. Williams, D. F. & Kohl, B. *Init. Rep. DSDP* **96**, 671-676 (1986).
13. Broecker, W. S. *Geochim. cosmochim. Acta* **46**, 1689-1705 (1982).
14. Broecker, W. S. & van Donk, J. *Rev. Geophys. Space Phys.* **8**, 169-198 (1970).
15. Emiliani, C. *J. Geol.* **63**, 538-578 (1955).
16. Brunner, C. A. *Quat. Res.* **17**, 105-119 (1982).
17. Brunner, C. A. & Cooley, J. F. *Geol. Soc. Am. Bull.* **87**, 681-686 (1976).
18. Jasper, J. P. & Gagosian, R. B. *Palaeoceanography* (in the press).
19. Fry, B., Jeng, W.-L., Scalan, R. S. & Parker, P. L. *Geochim. cosmochim. Acta* **42**, 1299-1302 (1978).
20. Roy Benedict, C., Wong, W. W. L. & Wong, J. H. H. *Pl. Physiol.* **35**, 512-517 (1980).
21. Snedecor, G. W. & Cochran, W. G. *Statistical Methods* (Iowa State University Press, 1978).
22. Deines, P. in *Handbook of Environmental Isotope Geochemistry* Vol. 1 (eds Fritz, P. & Fontes, J.) (Elsevier, Amsterdam, 1980).
23. Spiker, E. C. & Hatcher, P. G. *Org. Geochem.* **5**, 283-290 (1984).

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Sr isotope constraints on the Mediterranean environment at the end of the Messinian salinity crisis

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PROBABLY the most profound event that has affected the Mediterranean is the desiccation which occurred at the end of the Miocene[1–8]. During this period, there was a very rapid change from deep, open-marine conditions to environments of shallow-water carbonate and massive evaporite deposition[1–10]. Here we show that ostracod valves found in Messinian Lago Mare (Lake Sea) sediments, have a uniform but generally lower Sr isotopic composition than contemporary sea water thus providing evidence that some of the major Mediterranean basins (for example, Balearic and Levantine basins) formed large lacustrine systems that were probably interconnected towards the end of the Messinian salinity crisis[1–3] after the major evaporites were deposited. The most likely source of fresh water is from continental rivers flowing from the eastern European inland Paratethys sea or perhaps the Nile River. In the lower part of the Lago Mare, some ostracods have Sr isotopic compositions identical to contemporary sea water indicating a transient influence of marine-derived water. Sr compositions greater than that in contemporary sea water are only found in ostracods from onshore sediments in the Sorbas Basin[4,5] of southeastern Spain suggesting that this small basin, adjacent to the Straits of Gibraltar, became isolated after the deposition of the major evaporite sequences.

The events leading up to and during the so-called 'Messinian salinity crisis' have been the subject of diverse interpretations[6–11]. Here we discuss whether the various basins in the Mediterranean were interconnected during the Messinian, the source and quantity of continental drainage flowing into the Mediterranean during that period, whether the Paratethys connection was still open, and whether marine water inundated the Mediterranean at the end of the Messinian salinity crisis, when the Mediterranean consisted of a series of small lakes or a large lake, referred to as Lago Mare[6,8,11].

We assume[12] that the Sr isotopic composition of water masses isolated from the open oceans will, in part, reflect the composition of the continental drainages flowing into the closed basin. As the Sr isotopic composition of sea water is now precisely defined throughout the Tertiary[13–16] it is now possible to resolve even relatively small differences (parts per 10^5) from open-marine environments. In addition, the Sr isotopic composition in all present-day oceans (including the Mediterranean) are identical within analytical errors[13]. With the collision of Eurasia and Africa[17] and the progressive closure of the Tethys Ocean, the Mediterranean became separated from the eastern inland sea, Paratethys, which had previously provided an Indo-Pacific oceanic connection. The Mediterranean's last links to the open-marine environment were probably through the Betic and Rif Straits on the western side (adjacent to the present-day Straits of Gibraltar) which closed in the middle-late Miocene[18]. Thus, with its total isolation, together with the general trend to drier and cooler climate, the Mediterranean was destined for a 'salinity crisis'[1]. We expect this to be reflected in a change of the Sr isotopic composition in the Mediterranean lacustrine system relative to that of the open-marine Messinian sea water.

Ostracods are ubiquitous bivalved microcrustaceans that can live in continental, estuarine, marine and hypersaline waters[19]. Unlike molluscs and foraminifers, which continue to secrete additional layers or chambers, ostracods are arthropods and have to moult and build new valves after shedding the old ones[20]. The components of their low-Mg calcite shells are taken directly from the host water after moulting[21,22] and hence the Sr isotopic composition of the valves is an accurate monitor of the ambient composition of the aquatic environment. The particular ostracod we chose belongs to the well-known euryhaline genus *Cyprideis*. It is cosmopolitan and tolerates a wide range of salinities; modern representative of this genus can be found in fresh waters and in waters with salinities up to 100‰[23]. We analysed ostracods from several Deep-Sea Drilling Program (DSDP) sites (129A, 372 and 376) as well as two onshore sites in Spain and Cyprus. All samples are of Messinian age and postdate the evaporitic depositional phase of gypsum and/or halite, and thus date from very close to the Miocene/Pliocene boundary. We determined the Sr isotopic composition of carefully selected, unaltered single ostracod valves following the procedures outlined in ref. 12. A systematic study of the Sr/Ca ratios in the valves

(Table 1) indicated that diagenetic effects were minimal. We had to analyse single valves because of the extremely limited quantities of material available from the drill-core sites. Here the relevant comparison is with the $^{87}Sr/^{86}Sr$ ratio of sea water of the same date as the Miocene/Pliocene boundary, and hence the results are expressed in ΔSr units which represent deviations in parts per 10^4 from Messinian sea water[24].

The most complete section that has been studied is from DSDP site 376 which is in the Levantine Basin in the eastern Mediterranean. In core 376 the Sr ratios (Table 1 and Fig. 2) are relatively constant for the first 25 m just below the Pliocene/Miocene boundary (core depths of 55–80 m) with ΔSr values ranging from −3.8 to −5.2. In the same depth interval (level 6-4/101, which is equivalent to our sample M16) the controversial 'mystery sapropel' occurs. This consists of reworked giant *Orbulina* specimens together with other planktonic foraminifers and *Cyprideis* ostracods. The nearly constant ΔSr values for samples in this depth interval (55–80 m) is consistent with a sapropel consisting of locally reworked ostracods and foraminifers from slightly older layers. At ~100-m depth (~45 m below the Miocene/Pliocene boundary) the ΔSr values increase to values essentially identical to Messinian sea water ($\Delta Sr \sim 0$). This change in Sr isotopic compositions also coincides with a lithological transition from dolomitic marlstone with sapropelic layers to sandstone with interbedded marlstone. At that particular transition, truly freshwater ostracods (such as *Herpetocypris* and *Metacypris* juveniles, some *Candonopsis* and possibly *Ilyocypris*) were identified together with *Cyprideis*. The presence of these freshwater species excludes the presence of marine water during the lifetime of the ostracods, whereas the Sr isotopic composition indicates host water with a marine ancestry. This dilemma can be resolved by assuming that the ionic components of the early lacustrine water were themselves derived largely from evaporated brines of marine origin and thus obtained a (Miocene) marine Sr isotopic imprint. Alternatively there may have been a transient input of marine water which dominated the Sr isotopic budget for a short period but had little overall effect on the lacustrine environment.

At the base of core 376 (samples M33 and M40) intermediate ΔSr values of −2.8 to −2.5 are found together with Sr/Ca ratios[25] approaching seawater values, whereas at the top of the core, near the Miocene/Pliocene boundary, Sr/Ca ratios lower than contemporaneous sea water are present. Thus, the more negative ΔSr values found in the upper section of the core indicate a trend towards low-salinity continental-derived waters (but still NaCl dominant), whereas the less negative values lower in the section (just above the uppermost gypsum layers) must have been in contact with hypersaline solutions. The presence of marls together with sapropelic layers in the upper section of core 376 (core depth ~50–80 m) also indicates an obvious hydrological transition from salt precipitation in shallow salinas to further dilution by waters with a more obvious continental isotopic imprint.

We have also analysed ostracods from the DSDP core 129A collected in the western Levantine Basin. The ΔSr values fall in the narrow range −4.6 to −5.0 which is the same as that found

TABLE 1 Sr isotope data for Mediterranean Messinian ostracods

Sample	Sample core/section-interval (cm)	Depth* (m)	Sr/Ca†	$^{87}Sr/^{86}Sr$	$\Delta Sr(t)$‡
DSDP site 376					
M16	6-4/110-111	54.5	0.00268	0.708689 ± 32	−3.8 ± 0.5
M20 I	6-4/130-131	54.8	0.00296	0.708635 ± 27	−4.6 ± 0.4
II				0.708622 ± 31	−4.8 ± 0.4
III				0.708625 ± 29	−4.7 ± 0.4
M24 I	9-1/65-66	74.6	0.00237	0.708610 ± 28	−4.9 ± 0.4
II				0.708632 ± 29	−4.6 ± 0.4
M26	9-3/40-42	77.4	0.00286	0.708621 ± 25	−4.8 ± 0.4
M30 I	9-3/136-138	78.3	0.00280	0.708592 ± 26	−5.2 ± 0.4
II				0.708612 ± 29	−4.6 ± 0.4
M31 I	9-3/138-140	78.32	0.00276	0.708677 ± 30	−4.0 ± 0.4
II				0.708675 ± 29	−4.0 ± 0.4
M33 I	11-3/100-102	100	0.00271	0.708952 ± 34	−0.1 ± 0.4
II				0.708881 ± 19	−1.1 ± 0.3
M40 I	14/CC	131	0.00390	0.708762 ± 23	−2.8 ± 0.3
II				0.708781 ± 26	−2.5 ± 0.4
DSDP site 129A					
M34 I	2/CC			0.708606 ± 27	−5.0 ± 0.4
II				0.708606 ± 11	−5.0 ± 0.2
M3B I	2/CC			0.708629 ± 17	−4.7 ± 0.2
II				0.708633 ± 21	−4.6 ± 0.3
DSDP site 372					
M9 I	4-2/90-92	152	0.00320	0.708659 ± 10	−4.2 ± 0.2
II				0.708639 ± 28	−4.5 ± 0.4
III				0.708757 ± 14	−2.9 ± 0.2
Spain					
Dk75059 I			0.00464	0.709131 ± 23	2.4 ± 0.3
II				0.709066 ± 26	1.5 ± 0.4
III				0.709084 ± 22	1.7 ± 0.3
IV				0.709108 ± 21	2.1 ± 0.3
Cyprus					
GG-Cy-6 I			0.00293	0.708823 ± 23	−1.9 ± 0.3
II				0.708950 ± 20	−0.1 ± 0.3

Sr isotope ratios were measured on individual valves using techniques described in ref. 12. Measurements were made using a MAT261 with a value for NBS 987 = 0.710200 ± 10.

* True depth in core (from ocean floor).

† Mean Sr/Ca atomic ratios for individual horizons indicating that the ostracod valves did not undergo diagenesis. For further details see ref. 25.

‡ $\Delta Sr = ([^{87}Sr/^{86}Sr_{meas}(t)]/[^{87}Sr/^{86}Sr_{sea\ water}(t)] - 1)10^4$ where $t = 5$ Myr and $^{87}Sr/^{86}Sr_{sea\ water}(t) = 0.708960$ (ref. 24).

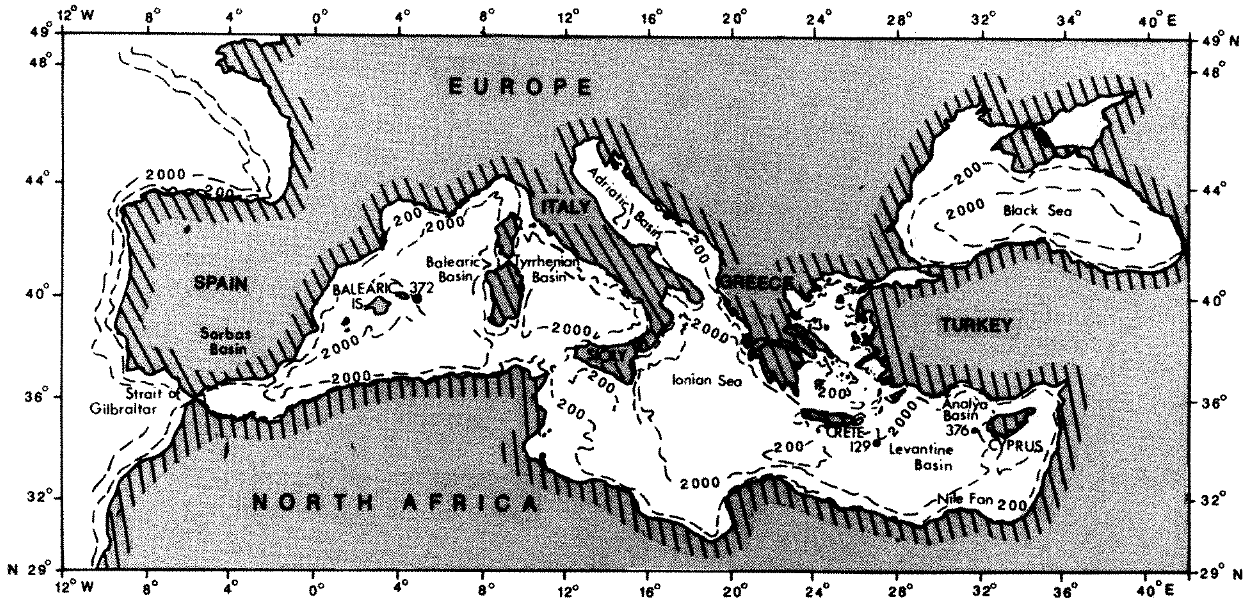

FIG. 1 Map of the Mediterranean Basin showing the location of the DSDP drill holes and the onshore sample sites. Ostracods from the Balearic Basin in the west and the Levantine Basin in the east have similar Sr isotopic compositions indicating the presence of a large (>2,000 km) interconnected lacustrine system.

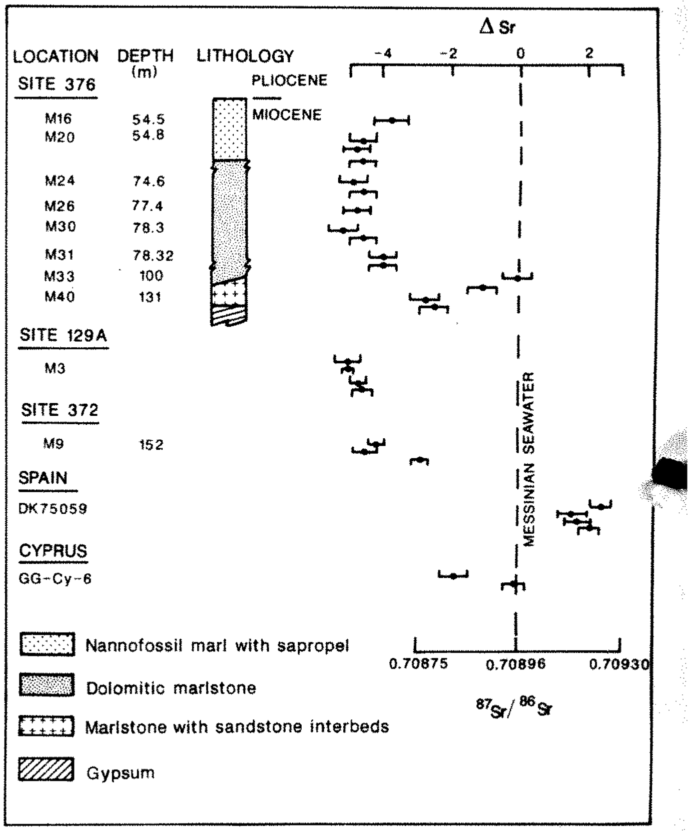

FIG. 2 Graph showing the Sr isotopic composition as a function of depth (in metres below the sea floor) for individual ostracod valves from several DSDP drill-core sites and onshore sites in the Mediterranean basin. The ΔSr values represent deviations in parts per 10^4 of the $^{87}Sr/^{86}Sr$ ratio from the value for Messinian sea water[24].

in the upper 80 m of DSDP site 376 from the same basin. In addition three valves were analysed from DSDP site 372 in the Balearic basin of the western Mediterranean (see Fig. 1). Two of the valves have negative ΔSr values falling within the same narrow range (−4.2 to −4.5) as found in the upper section of sites 376 and 129A from the Levantine Basin. The slightly discordant value of -2.9 ± 0.2 (sample M9C) is identical to that found at the base of core 376. The similar and limited range of negative ΔSr values provides strong evidence for a direct connection between some of the major Messinian, Lago Mare Basins (that is, the Balearic and Levantine Basins), despite the fact that they were separated by distances of over 2,000 km. It is therefore likely that the inland sea, the Paratethys, must have extended as far as the Mediterranean Basin.

The two onshore samples of Messinian age from Cyprus[25,26] also have negative ΔSr values; one value is identical to that Messinian sea water. This is within the same range of values as observed at the base of core 376. In contrast to these results, ostracods from the Messinian of southeastern Spain from the 'white lagoonal limestone' (that is, the white layer which represents the terminal Messinian)[4,5,27] of the Sorbas Basin have positive ΔSr values of 1.5 to 2.4. These values are markedly different from those of the major Lago Mare Basins and clearly imply that the Sorbas Basin had a different continental input compared to the major Mediterranean Basins, including the adjacent Balearic Basin. The distinctive ΔSr values indicate, contrary to what is observed in the other Messinian Mediterranean basins, that the Sorbas Basin was a restricted (barred) basin isolated from the rest of the Mediterranean after deposition of the gypsum evaporitic beds. The Sr/Ca ratios in ostracods from the Sorbas Basin form a broad range; some of the values are different from typical Messinian marine waters. This further confirms the independent conclusion[27] based on the Zorreras Member from the Sorbas Basin (within which the white lagoonal limestone occurs) which was deposited at the time of closure of the Betic Straits.

Our results for sites 376 and 129A in the Levantine Basin, site 372 from the Balearic Basin and the onshore samples from Spain and Cyprus, have a number of important implications for the palaeoenvironment of the Messinian Mediterranean. The exceedingly narrow range of negative ΔSr values provides good evidence for a direct connection between the widely separated large basins of the Lago Mare in the Mediterranean, immediately before the Pliocene transgression. The Sorbas Basin on the western edge of the Mediterranean seems to be an exception. These observations are consistent with the deep-basin model[1-4,28] of the Messinian Mediterranean, in which a large 'inland sea' (the Lago Mare[6,8,11]) formed that was largely isolated from the open-marine environment at the end of the Messinian. Thus the 'wet' Mediterranean theory[29] may have some relevance for the Lago Mare phase, but it is clear that a lacustrine, not a marine, environment existed. A lacustrine environment close to freshwater is indicated by the Mg/Ca ratios *Cyprideis* ostracods[25] as well as by the presence of truly freshwater ostracod taxa.

The obvious lithological change from sandstones to marls with sapropelic layers coinciding with low Sr/Ca ratios (Table 1) and a change in ΔSr values of the ostracod shells, indicates an influx of fresh (or brackish) continental water at site 376. It has been proposed[1] that the source of this water is the inland Paratethys Sea of eastern Europe. To account for the negative ΔSr values the inflowing waters must also have ΔSr values as, or more negative, than those observed. This could be the result of dissolution of Sr from young volcanic provinces or, more probably, from older limestones. From the Sr-isotope seawater curve[13-15], lower Tertiary or limestones as old as Ordovician would be suitable sources. Another possible source is the Nile River. At present the Nile has a very negative ΔSr value ($\Delta\text{Sr} \sim -60$)[30,31] and its waters could rapidly lower the Sr isotopic composition of the Mediterranean Lago Mare lacustrine waters. The narrow range of only slightly negative ΔSr values that has been observed for the widely separated sites, 376 and 372, however, suggests that it is unlikely that Nile River water was a significant input to the Mediterranean Basin. The seawater-like ΔSr values at the bottom of core 376 and in the Cyprus sample, together with the presence of ostracods of lacustrine affinity, may be due to a transient marine influx but is more probably due to redissolved, evaporated brines containing Sr that had a marine origin. Throughout this period (latest Messinian) the ostracods indicate that the waters in the Lago Mare remained NaCl-dominant (that is, chemical pathway II [32]), even in their dilute stage, as shown by the presence of *Cyprideis* ostracods which have a preference for this type of water[33]. □

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1. Hsü, K. J. *et al. Nature* **267,** 399–403 (1977).
2. Hsü, K. J. *et al. Init. Rep. DSDP* Leg **42,** 219–304 (1978).
3. Melières, F., Chamky, H., Coumes, F. & Rouge, P. *Init. Rep. DSDP* Leg **42,** 361–384 (1978).
4. Roep, T. B. & van Harten, D. *Annls Géol. Pays Hell. Tome hors serie* **III,** 1037–1044 (1979).
5. Dronkert, H. *Memorie Soc. geol. ital.* **16,** 341–361 (1976).
6. Cita, M. B., Wright, R. C., Ryan, W. B. F. & Longinelli, A. *Init. Rep. DSDP* Leg **42,** 1003–1035 (1978).
7. Drooger, C. W. (ed.) *Messinian Events in the Mediterranean* (North Holland, Amsterdam, 1973).
8. Hsü, K. J. *et al. Init. Rep. DSDP* Leg **42,** 1–1249 (1978).
9. McKenzie, J. A. & Ricchiuto, T. E. *Init. Rep. DSDP* Leg **42,** 650–655 (1978).
10. Pierre, C. & Fontes, J. C. *Init. Rep. DSDP* Leg **42,** 635–650 (1978).
11. Ruggieri, G. *Publs Syst. Ass.* **7,** 283–290 (1967).
12. McCulloch, M. T., De Deckker, P. & Chivas, A. R. *Geochim. cosmochim. Acta.* **53,** 1703–1710 (1989).
13. DePaolo, D. J. & Ingram, B. L. *Science* **227,** 938–941 (1985).
14. Palmer, M. R. & Elderfield, H. *Nature* **314,** 526–528 (1985).
15. Koepnick, R. B. *et al. Chem. Geol.* **58,** 55–81 (1985).
16. Elderfield, H. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **57,** 71–90 (1986).
17. Mulder, C. J. *Messinian Events in the Mediterranean* (ed. Drooger, C. W.) 44–59 (North Holland, Amsterdam, 1973).
18. Weijermars, R. *Tectonophysics* **148,** 211–219 (1988).
19. De Deckker, P., Colin, J. P. & Peypouquet, J. P. (eds) *Ostracoda in the Earth Sciences* (Elsevier, Amsterdam, 1988).
20. De Deckker, P. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **62,** 463–475 (1988).
21. Turpen, J. B. & Angell, R. W. *Biol. Bull.* **140,** 331–338 (1971).
22. Chivas, A. R., De Deckker, P. & Shelley, J. M. G. *Hydrobiologia* **143,** 135–142 (1986).
23. De Deckker, P. *Hydrobiologia* **81,** 131–144 (1981).
24. McKenzie, J. A., Hodell, D. A., Mueller, P. A. & Mueller, D. W. *Geology* **16,** 1022–1025 (1988).
25. De Deckker, P., Chivas, A. R. & Shelley, J. M. G. *Palaios* **3,** 352–358 (1988).
26. Rouchy, J. M. *Bull. Centre Rech. Expl Elf-Aquit.* **4,** 511–545 (1980).
27. Müller, D. W. & Hsü, K. J. *Paleoceanography* **2,** 679–696 (1987).
28. Hsü, K. J. *Scient. Am.* May 53–63 (1978).
29. Dietz, R. S. & Woodhouse, M. *Geotimes* **43,** 4 (1988).
30. Brass, G. W. *Geochim. cosmochim. Acta* **40,** 721–730 (1976).
31. Palmer M. R. & Edmond J. M. *Earth planet. Sci. Lett.* **92,** 11–26 (1989).
32. Eugster, H. P. & Hardie, L. A. in *Saline Lakes* (ed. Lerman, A.) 237–294 (Springer, Berlin, 1978).
33. Anadon, P., De Deckker, P. & Julia, R. *Hydrobiologia* **143,** 199–208 (1986).

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Hydrothermal oil of Guaymas Basin and implications for petroleum formation mechanisms

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PETROLEUM-LIKE hydrocarbons have been detected in thermally altered Recent sediments of Guaymas Basin[1-5] and petroleum-like hydrocarbon impregnations were found in hydrothermal mounds on the sea floor and associated with hydrothermal vent emissions[5-9]. Here we report the evaluation of such a hydrothermal oil, which we find to be similar to conventionally exploited crude oils. Its young geological age (<5,000 yr, ^{14}C)[10] indicates that a significant fraction of the organic carbon in the oil has completed the transformation from biomass to migrating oil in less than 5,000 years, thus limiting the oil generation, explusion and migration processes to a geologically short timescale. We estimate the generation potential of such hydrothermal oil and discuss its implications to our understanding of the petroleum generation, expulsion and migration mechanisms.

Guaymas Basin, located in the central part of the Gulf of California, is part of a rift zone that links the East Pacific Rise in the south with the San Andreas Fault leading north. The tectonic graben-like basin is covered by thick layers of sediments (up to ~500 m), consisting of diatomaceous ooze and terrigenous silty mud[11,12]. Hydrothermal activity in the area was postulated on the basis of anomalously high heat flow[11,13,14] and then detected by instrument-assisted observations[15]. Later, diving with the submersible *Alvin* allowed direct sampling and gathering of visual evidence on the hydrothermal systems of Guaymas Basin[16-18].

The hydrothermal activity in Guaymas Basin releases[17,19-21] discrete oil globules (up to 1-2 cm in diameter), which are discharged into the water column with the hydrothermal fluids. The disturbance of the surface layers of bottom sediments and chimney debris also releases oil globules. Direct measurement of the oil flow rate at the vent sites has so far not been feasible, but the *in situ* collection of oil globules showed that the gas/oil ratio is ~5, with possible gas dissolution in hydrothermal water and seawater exposure of the oil before and during expulsion from the vent system[22]. Low bottom temperatures (2-3 °C) contribute to condensation and retention of some of the oil and this generates oil impregnations on and in inorganic substrates of the hydrothermal vent systems.

A hydrothermal chimney (40-cm length × 8-12 cm diameter; ~1,500 g), was removed from a vent that was releasing hydrothermal fluids (translucent water, at ~200 °C) at the moment of sampling (DSV *Alvin* Dive 1630, Fig. 1 insert) at 2,020-m water depth[20,22]. At the surface the sample was sealed in double, pre-washed Kapak bags and frozen (−40 °C) until analysis.

The hydrothermal chimney consisted of a porous unconsolidated inorganic matrix (57.0%), water (19.2%) and a high organic matter content (23.8%). On thawing the sample released 2.1% of free-flowing oil. The vapour phase in contact with the sample was analysed for light hydrocarbons and alkanes from methane (15.5%) to hexadecane (0.7%) were detected. Selected hydrocarbon ratios[22] are similar to those of the gasoline fraction of typical crude oils. The general distribution pattern of light volatile hydrocarbons in this chimney resembles that of crude oils, with the exception of a lower content of aromatic hydrocarbons. This is probably caused by water washing and results from the sample-water contact during formation of the chimney, sample collection and retrieval from the sea floor (~2,000-m depth). No olefins were detected among the volatile hydrocarbons indicating that the organic matter had not been exposed to severe thermal cracking processes. The inorganic matrix of the chimney consisted predominantly (85%) of barium sulfate crystals[23], with the organic matter adsorbed and impregnating the interstitial pore space of the unconsolidated barite matrix.

The hydrothermal oil from the Guaymas Basin is similar to reservoir crude oils. First, the total extractable organic matter distilled over the normal range, but the gasoline fraction is low indicating that a significant portion of the volatile (C_1–C_{10}) components were lost in the thermocline before recovery and preservation. Second, the elemental composition (Table 1*a*) is within the normal ranges of typical crude oils[24], with slightly higher contents of vanadium and nickel as we expect for a light oil with a relatively high content of intermediate distillation products. Third, the porphyrin and biomarker contents and their distribution in this sample are well within the range of normal crude oils[25], indicating moderate degrees of maturation and thermal stress. Fourth, the compound-class composition (Table 1*a*) is just like a typical reservoir crude.

TABLE 1 Hydrothermal petroleum from Guaymas Basin

(*a*) *Characteristics*

Characteristics of organic matter	
Specific gravity (60 °F/60 °F)	0.8891
API gravity (60 °F)	27.6
Conradson carbon	4.2%
Carbon	85.0%
Hydrogen	11.1%
Nitrogen	0.49%
Oxygen	1.50%
Sulphur	0.99%
Phosphorus	0.005%
Vanadium	214 p.p.m.
Nickel	98 p.p.m.
Ratios (atomic)	
Carbon/Hydrogen (C/H)	0.64 (H/C = 1.57)
Carbon/Nitrogen (C/N)	142
Carbon/Oxygen (C/O)	75
Carbon/Sulphur (C/S)	228
Carbon/Phosphorus (C/P)	44,250
Vanadium/Nickel (V/Ni)	2.56

Distillation Yield		
Gasoline (IBP–300 °F)		3%
Kerosene–Diesel (300–600 °F)		61%
Gas oil (660–1,000 °F)		21%
Residue (>1,000 °F)		15%
Composition*	**Free oil**	**Total extract**
Paraffinic & naphthenic HC	54.5%	44.6%
Aromatic HC	26.6%	34.6%
N, S & O compounds	9.8%	8.8%
Asphaltenes	9.1%	12.8%

(*b*) *Biomarkers*

		Comments		
		Structural distribution	Carbon number	Relative molecular mass distribution
I	Metallo porphyrins type Vanadyl 363 p.p.m. Nickel 145 p.p.m.	ETIO† > 95% DPEP‡ < 5%§	C_{24}–C_{30} max C_{27}	366–450 max 408
II	n-Alkanes Range n-C_{12}–n-C_{25} max n-C_{15}	Carbon preference index $C_{11\text{-}22} = 0.99$	Monomodal	170–352
III	n-Alkylcyclohexanes C_{13}–C_{22} max C_{17}		Monomodal	182–308
IV	n-Alkylbenzenes C_{13}–C_{22} max C_{17}		Monomodal	176–302
V	Isoprenoids C_9–C_{20} max C_{19}	Pristane/Phytane 1.85		128–282
VI	Terpanes‖ C_{19}–C_{35} max C_{30}	Tricyclic C_{19}–C_{29} Pentacyclic C_{27}–C_{35}		260–482
VII	Steranes/Diasteranes¶ C_{21}–C_{29} max C_{27}			288–400
VII	Unresolved complex mixture n-C_{12}–C_{25} (GC retained) max n-C_{17} (GC retained)		Monomodal	158–348

* >200 °C fraction of oil.

† ETIO, etioporphyrins.

‡ DPEP, deoxophylloerythroetioporphyrins.

§ Conversion of DPEP to ETIO porphyrins is associated with the thermal evolution of geolipids[31] and is also associated with a decrease in porphyrin concentration and displacement of the porphyrin-carbon-number maximum towards lower values[32].

‖ Other biomarkers in the hydrocarbon fraction consist of triterpenoids, extended tricyclic terpanes and steranes with their rearranged analogues (diasteranes)[6,8,9,33]. Minor extended tricyclic terpanes range from C_{20} to C_{29} with a similar distribution pattern as observed for other mature petroleum samples. The triterpanes are essentially 'mature' in their more stable configurations and are comprised primarily of the 17α(H), 21β(H)-hopane series ranging from C_{27} (no C_{28}) to C_{35}, with the homologues from C_{31}–C_{35} present as 22-*S* and *R* epimer pairs in a ratio of about 1.3 (*S*/*R*)[6,8]. Minor amounts of 17β(H), 21α(H)-hopanes (moretanes, C_{29}, C_{30} and C_{31}) are also present.

¶ The steroidal markers primarily consist of 5α(H), 14α(H), -17α(H)-steranes (20*R*), and diasteranes mainly as the 13β(H), 17α(H)-20 *R* or *S* series. The steranes range from C_{26} to C_{30}, with cholestane as the major homologue. The distribution pattern indicates a marine autochthonous origin, an intermediate maturity and is comparable with data for other samples from the area[6,8].

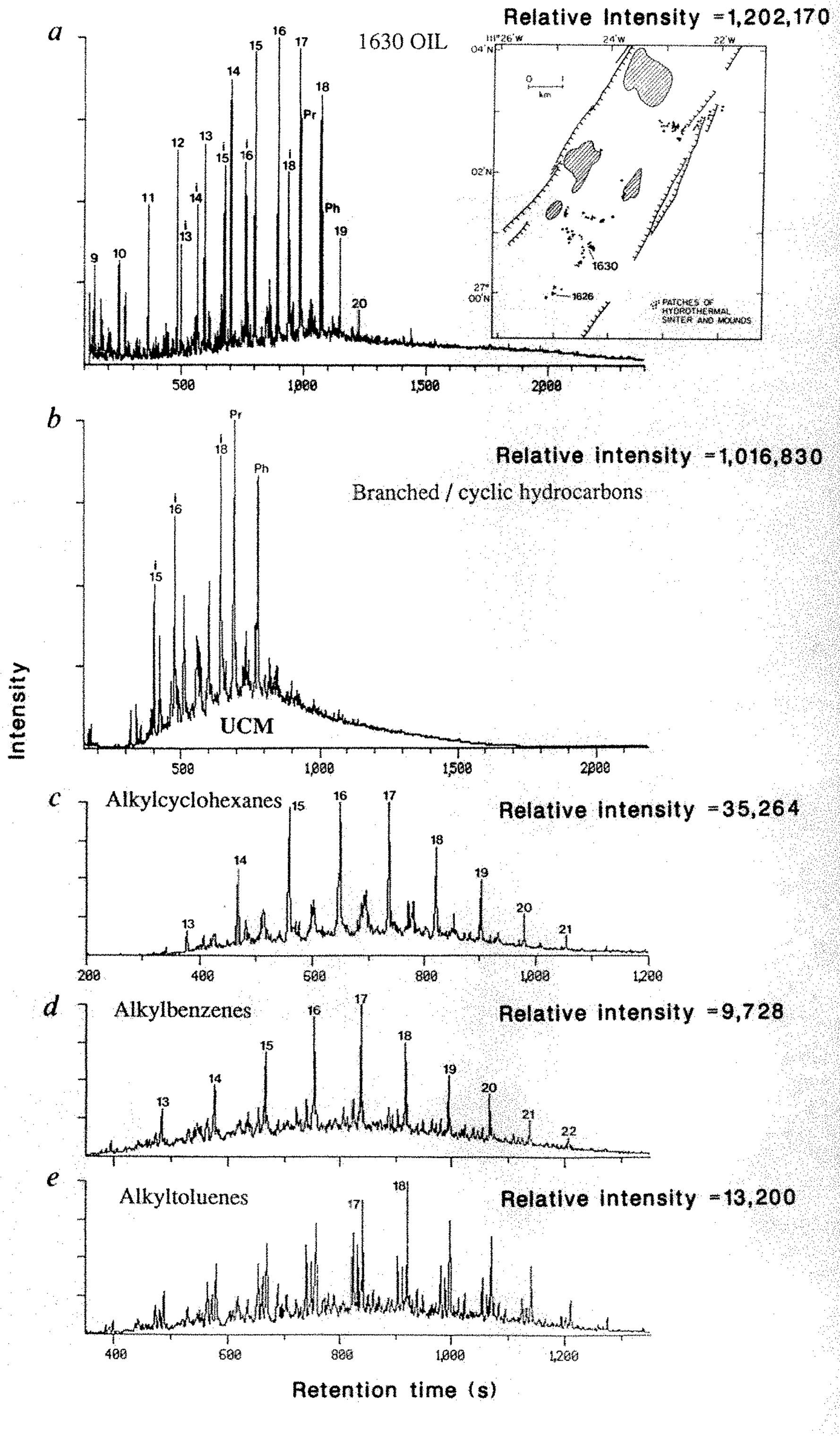
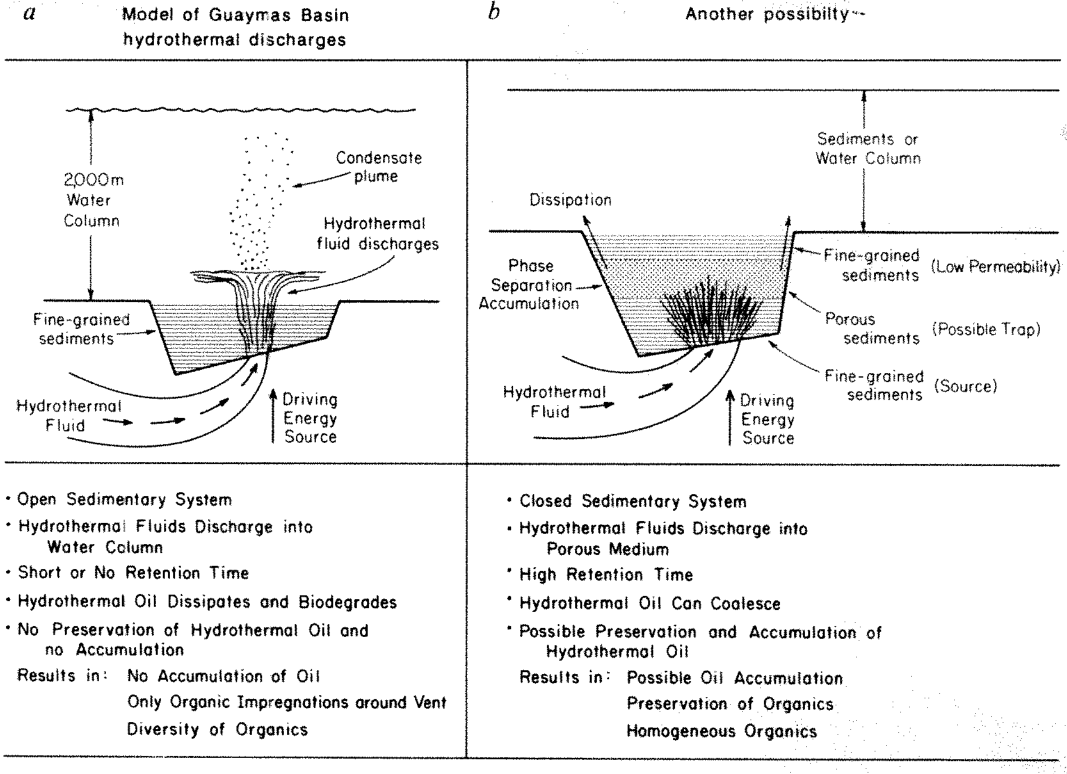


FIG. 1 Salient features of the gas chromatography/mass spectrometry data for the *a*, total and the *b*–*e*, branched-cyclic hydrocarbons of oil sample 1630 (inset in *a* location map of the dive area in the south rift of Guaymas Basin). *a*, Total ion-current trace (numbers refer to *n*-alkanes, i *n* to isoprenoid hydrocarbons, Pr to pristane, Ph to phytane). The *n*-alkane range for the stabilized oil (C_{12}–C_{24}, max C_{15}) has a monomodal, narrow carbon-number distribution with no carbon preference (carbon preference index C_{12}–C_{22}=0.99), compatible with a predominantly bacterial/algal origin for the source organic matter and with an adequate degree of maturation. *b*, Total ion-current trace (i *n*, isoprenoid hydrocarbons; UCM, unresolved complex mixture, that is, naphthenes). A range of isoprenoid hydrocarbon biomarkers is present, C_{15}–C_{20} (no C_{17}) and pristane as the dominant homologue. *c*, $m/z=83$, key ion for alkylcyclohexanes (numbers indicate carbon number of homologues). *d*, $m/z=91$, key ion for alkylbenzenes. *e*, $m/z=105$, key ion for alkyltoluenes. On the whole, these distributions are compatible with an algal/bacterial origin for the organic matter.

The gas chromatography/mass spectrometry data (Fig. 1) are compatible with a predominantly bacterial/algal origin for the source organic matter and suggest an adequate degree of maturation.

Sediments have been accumulating in the rift areas of Guaymas Basin for ~150,000 yr at an assumed rate of 0.2 cm yr^{-1} and this, combined with turbidite flows, has resulted in a ~400-m layer of diatomaceous ooze[11]. Thus, if the oil associated with these vent systems has not originated outside the rift areas and is, as indicated by previous geological and geochemical evidence[11,17], autochthonous from the Guaymas Basin sediments, it has probably formed by the action of hydrothermal processes on the organic matter of these relatively Recent sediments[4,5,7]. Radioactive-carbon isotope (^{14}C) dating of the oil from this and a nearby area (dives 1630 and 1626) gives ages of 4.2-4.9 × 10^3 yr with uncertainties in the range 50-190 yr (ref. 10). Therefore, in Guaymas Basin, the overall time-temperature conversion of sedimentary organic matter to hydrothermal petroleum takes place over a short geological timescale (<5,000 yr) and commences under relatively mild temperature conditions, as indicated by the presence and characteristics of the biomarkers. The oil expulsion and migration mechanisms are provided by the hydrothermal fluids under pressures of 200 bars and temperature conditions up to and exceeding 315 °C


a
Model of Guaymas Basin hydrothermal discharges
2,000 m Water Column
Condensate plume
Hydrothermal fluid discharges
Fine-grained sediments
Hydrothermal Fluid
Driving Energy Source
b
Another possibilty--
Sediments or Water Column
Dissipation
Phase Separation Accumulation
Fine-grained sediments (Low Permeability)
Porous sediments (Possible Trap)
Fine-grained sediments (Source)
Hydrothermal Fluid
Driving Energy Source

- Open Sedimentary System
- Hydrothermal Fluids Discharge into Water Column
- Short or No Retention Time
- Hydrothermal Oil Dissipates and Biodegrades
- No Preservation of Hydrothermal Oil and no Accumulation

Results in: No Accumulation of Oil
Only Organic Impregnations around Vent
Diversity of Organics

- Closed Sedimentary System
- Hydrothermal Fluids Discharge into Porous Medium
- High Retention Time
- Hydrothermal Oil Can Coalesce
- Possible Preservation and Accumulation of Hydrothermal Oil

Results in: Possible Oil Accumulation
Preservation of Organics
Homogeneous Organics

FIG. 2 Schematic models for hydrothermal petroleum generation and migration scenarios: *a*, Guaymas Basin open system (in excess of ~200 bar). *b*, Hypothetical closed system.

at some vent outlets. There are probably near-critical conditions further down in the sedimentary column[8,26,27].

The hydrothermal petroleum is discharged into the water column, where it is exposed to water washing, dispersion and active biodegradation. This is supported by the compositional data for petroleum impregnations with different degrees of environmental exposure and biodegradation in the Guaymas Basin area[6-8]. Thus, hydrothermal-oil transport and release results in its dispersion, alteration and eventual loss. A schematic representation of the prevailing conditions in the Guaymas Basin systems[28] is shown in Fig. 2*a*. Hydrothermal fluids driven by a deep heat source, permeate through an open, fine-grained body of Recent sediments and discharge directly into the water column. The oil discharged with the hydrothermal fluids partially adsorbs or condenses on inorganic substrates cooled by sea water (~3 °C) surrounding the vents. The major part of the oil plume above the vent area dissipates into the water column mainly by dispersion, dissolution and eventual biodegradation. Another scenario could be postulated (for example, Fig. 2*b*) where a similar hydrothermally generated oil is discharged into a porous sedimentary body, with a finite retention time for the fluids. There the hydrothermal oil-water mixtures can undergo phase separation and petroleum could eventually accumulate if adequate sedimentary and tectonic features are available.

TABLE 2 Hydrothermal petroleum potential of Guaymas Basin (South Trough)

Basic considerations used for estimate:	
Area of hydrothermal activity presently known	~3 × 10 km = 30 km^2
Sedimentary layer	~300–500 m* = 400 m
Hydrothermally altered sedimentary layer	lower 120 m* = 120 m
Porosity	40–80%* = 50%
Organic carbon (dry weight, unaltered section)	av. 2%† = 2%
Organic carbon (altered section)	decreases to 0.2%† = 0.2%
Estimates:	
Sediment volume (dry basis)	= 2 × 10^8 m^3 km^{-2}
Sediment weight (dry, used density 1 g cm^{-3})	= 2 × 10^8 t km^{-2}
Organic carbon converted (2% to 0.2% = 1.8%)	= 1.08 × 10^6 t OC km^{-2}
Organic matter (oil = 85% TOC)	= 1.3 × 10^6 t oil km^{-2}
Total oil in altered section of area (30 km^2)	= 40 × 10^6 t

* Compare to data in ref. 11.
† Compare to data in ref. 32.

In Guaymas Basin the presently known area where hydrothermal activity reaches the sea floor is ~3 × 10 km and the lower 120-m section of the sediments drilled was hydrothermally altered[11]. An average porosity of ~50% and an organic carbon decrease from 2.0 to 0.2% (typical of the area[29]) give a value for total organic matter (oil potential) susceptible to hydrothermal alteration and fluid leaching of the order of 1.3 × 10^6 t km^{-2} of alteration zone (Table 2). This gives a maximum organic-matter availability of ~40 × 10^6 t for the presently known hydrothermally active area of the basin (Table 2). So far no data is available on the ultimate efficiency of hydrothermal petroleum generation processes prevailing in Guaymas Basin. The combination of field data with a conservative potential-yield calculation, however, indicate a high efficiency and significant quantities of hydrothermal oil could have been generated by this relatively small area during its geologically active lifetime. If larger areas and volumes of sediments were involved (where there is, at present, no evidence that hydrothermal activity reaches the sea bottom or if larger hydrothermally altered areas have formed because of hot-spot displacement along the tectonic axis of the graben during its geologic evolution) the overall hydrothermal-petroleum generating potential of the area could be considerably larger.

The generally accepted model of oil generation, postulated to be responsible for the formation of the major part of the presently exploited petroleum, assumes long-term maturation of sedimentary organic matter in subsiding sedimentary basins, where the organic matter undergoes successive and gradual increases in geothermal alteration leading to a process of continuous oil generation. The expulsion of the oil and its subsequent migration occur later and only after the geothermal maturation of the original organic matter. This multistep oil formation process has a low efficiency and converts only a minor fraction of the original organic matter of the sediment to oil. There is also difficulty in balancing and timing an adequate degree of geothermal maturation (oil generation) occurring at intermediate stages of basin evolution and ample fluid availability (expulsion), with the retention of sufficient intergrain porosity for adequate fluid transport (migration).

Although considerable progress in the understanding of this multistep oil formation mechanism has been achieved, there are still problems that need to be solved[30]. Such a slow multistep mechanism differs significantly from hydrothermal petroleum formation, which can be geologically fast (here 5,000 yr) indicating a single-step mechanism in which generation occurs almost concurrently with expulsion and migration. In addition the whole hydrothermal process can take place during the early stages in the geological history of a basin.

Although hydrothermal oil formation has been detected, identified and documented, no evidence is so far available on the extent to which this alternative single-step oil generation process has contributed towards the origin of presently exploited oil reserves. As no hydrothermal-oil reservoirs have yet been identified, a trapping mechanism is not known, but could proceed according to the scenario described in Fig. 2*b*. Nevertheless, hydrothermal oil generation occurs and could result in oil accumulations under favourable geological constraints. Hydrothermal oil formation provides an efficient single-step mechanism for petroleum generation, expulsion and migration which could have a considerable impact on our understanding of petroleum formation mechanisms and eventually assist us in tapping resources in new areas. ☐

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1. Galimov, E. M., Kodina, L. A., Bogacheva, M. P. & Shirinsky, V. G. *Init. Rep. DSDP* **64**(II) 819–836 (1982).
2. Simoneit, B. R. T. & Philp, R. P. *Init. Rep. DSDP* **64**(II) 883–904 (1982).
3. Simoneit, B. R. T., Mazurek, M. A., Brenner, S., Crisp, P. T. & Kaplan, I. R. *Deep-Sea Res.* A**26**, 879–891 (1979).
4. Simoneit, B. R. T. in *The Role of Heat in the Development of Energy and Mineral Resources in the Northern Basin and Range Province, Geotherm. Res. Coun., Spec. Rep. 13*, 215–241 (Davis, California, 1983).
5. Simoneit, B. R. T., Philp, R. P., Jenden, P. D. & Galimov, E. M. *Org. Geochem.* **7,** 173–205 (1984).
6. Kawka, O. E. & Simoneit, B. R. T. *Org. Geochem.* **11,** 311–328 (1987).
7. Simoneit, B. R. T. in *Hydrothermal Processes at Seafloor Spreading Centers* (eds Rona, P. A. *et al.*) 453–474 (Plenum, New York, 1984).
8. Simoneit, B. R. T. *Can. J. Earth Sci.* **22,** 1919–1929 (1985).
9. Simoneit, B. R. T. & Lonsdale, P. F. *Nature* **295,** 198–202 (1982).
10. Peter, J. M., Kawka, O. E., Scott, S. D. & Simoneit, B. R. T. *Third Chem. Congr. North America, Toronto* abstr. GEOC 036 (1988).
11. Curray, J. R. *et al. Init. Rep. DSDP* **64**(I, II) (1982).
12. Koski, R. A., Londsale, P. F., Shanks, W. C., Berndt, M. E. & Howe, S. S. *J. geophys. Res.* **90,** 6695–6707 (1985).
13. Lawver, L. A. & Williams, D. L. *J. geophys. Res.* **84,** 3465–3478 (1979).
14. Williams, D. L., Becker, K., Lawver, L. A. & von Herzen, R. P. *J. geophys. Res.* **84,** 6757–6796 (1979).
15. Lonsdale, P. F. *Eos* **61,** 995 (1980).
16. Lonsdale, P. & Lawver, L. A. *Geol. Soc. Am. Bull.* **91,** 555–591 (1980).
17. Lonsdale, P. *Am. Ass. Petrol. Geol. Bull.* **69,** 1160–1180 (1985).
18. Lonsdale, P. & Becker, K. *Earth planet. Sci. Lett.* **73,** 211–225 (1985).
19. Merewether, R., Olsson, M. S. & Lonsdale, P. *J. geophys. Res.* **90,** 3075–3085 (1985).
20. Gieskes, J. M. *et al. Can. Mineralogist* **26,** 589–602 (1988).
21. Simoneit, B. R. T. *Can. Mineralogist* **26,** 827–840 (1988).
22. Simoneit, B. R. T., Kawka, O. E. & Brault, M. *Chem. Geol.* **71,** 169–182 (1988).
23. Didyk, B. M. & Simoneit, B. R. T. *Third Chem. Congr. North America, Toronto* abstr. GEOC 035 (1988).
24. Tissot, B. P. & Welte, D. H. *Petroleum Formation and Occurrence: A New Approach to Oil and Gas Exploration* 2nd edn (Springer, 1984).
25. Baker, E. W. & Louda, J. W. *Org. Geochem.* **10,** 905–914 (1986).
26. Bischoff, J. L. & Rosenbauer, R. *J. Earth planet. Sci. Lett.* **68,** 172–180 (1984).
27. Chen, C.-T. A. *Science* **211,** 298 (1981).
28. Scott, S. D. in *MTS-IEEE Conf. Proc. Oceans 83*, vol. 2 818–824 (IEEE, San Francisco, 1983).
29. Simoneit, B. R. T. & Bode, G. R. in *Init. Rep. DSDP* **64**(II) 1303–1306 (1982).
30. Durand, B. *Org. Geochem.* **13,** 445–459 (1988).
31. Didyk, B. M., Alturki, Y. I. A., Pillinger, C. T. & Eglinton, G. *Nature* **256,** 563–565 (1975).
32. Didyk, B. M. thesis, Univ. Bristol (1975).
33. Didyk, B. M. & Simoneit, B. R. T. *App. Geochem.* (in the press).

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Evidence for anoxygenic photosynthesis from the distribution of bacteriochlorophylls in the Black Sea

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THE contribution of anoxygenic photosynthesis to carbon cycling in the Black Sea, the world's largest body of anoxic marine water, has been vigorously investigated and debated for over four decades[1–6]. Penetration of light into the sulphide-containing deep water may result in a zone of anaerobic primary production by photosynthetic bacteria. We report here the results of analyses of photosynthetic pigments in samples of suspended particulate matter collected from two stations in the western basin of the Black Sea. Our data demonstrate high concentrations of a bacteriochlorophyll at the chemocline, and thus the potential for anoxygenic photosynthesis as a component of primary production in the carbon cycle of the Black Sea[6]. More than 95% of the pigments in the bacteriochlorophyll-maximum are accounted for by a series of aromatic carotenoids and bacteriochlorophylls-*e*, including a previously unreported geranyl ester of 4-*i*-butyl bacteriochlorophyll-*e*. The distribution of pigments is characteristic of the obligate phototrophs *Chlorobium phaeobacteroides* and *C. phaeovibriodes*. Total depth-integrated bacteriochlorophyll at one station exceeded total chlorophyll-*a* in the overlying oxygenated portion of the euphotic zone. We suggest that anoxygenic photosynthesis is a relatively recent phenomenon in the Black Sea initiated by shallowing of the chemocline over the past decade and development of an anoxic layer devoid of O_2 and H_2S (ref. 7).

Suspended particulate matter was collected on RV *Knorr* cruise 134, leg 9 (Instanbul to Istanbul, Turkey) in May 1988. Station BS2-1 (42°10′N, 30°00′E) is located 110 km from the Turkish coast in 2,110 m of water, and is most heavily influenced by terrigenous material advected from the shelf-slope region of the adjoining coast. Dissolved oxygen was maximal (320 μM, 5% supersaturation) at 20 m and rapidly decreased to <2 μM (limit of detection) at depths >80 m. Dissolved sulphide was detected in all the samples that were collected below 100 m. Station BS2-2 (42°50′N, 32°00′E) is located in the centre of the western basin and is characterized by a shallower chemocline. Dissolved oxygen decreased from a maximum of 307 μM at 20 m to <2 μM at >56 m. Sulphide was detected at depths as shallow as 80 m (Fig. 1). The distributions of photosynthetic pigments in the oxic portion of the water columns at each station were similar (Table 1). The major pigments included: chlorophyll-*a*, chlorophyll-c_1 and chlorophyll-c_2, which are widely distributed in marine algae; chlorophyll-c_3, 19′-butanoylfucoxanthin and 19′-hexanoylfucoxanthin, which are characteristic of prymneseophytes[8,9]; and fucoxanthin, which is widely distributed but characteristic of algae from the class Bacillariophycae[10] (Fig. 2).

Particulate matter samples collected from 90–121 m (station BS2-1) and 60–110 m (station BS2-2) all contained abundant bacteriochlorophylls and bacteriocarotenoids. Four components were isolated that had visible absorption maxima in acetone of 463 and 653 nm. The main component (45% of the total) and two lesser components (16 and 15% of the total) were identified, respectively, as the 4-ethyl, 4-*n*-propyl and 4-*i*-butyl (Fisher nomenclature) homologues of bacteriochlorophyll-*e* by mass spectrometry and comparison of chromatographic retention

TABLE 1 Concentration of main caroteroids and chlorophylls

Depth (m)	Fxn	19'-HFxn	Chl-*a*	GBChl	EBChl	PBChl	BBchl	IR	β-IR
Station 1, 27 May 1988									
10	72	121	335						
20	94	89	342						
30	99	36	322						
40	71	18	255						
50	8		58						
60	37		88						
70	73		20						
90	18		12	23	39	15	15	17	9
100	6		15	53	89	35	37	20	8
106	10		15	11	26	15	13	7	5
112	6		15	9	16	10	11	4	3
121	19		17	7	17	10	9	2	1
140	3		12					2	1
Station 2, 17 May 1988									
2	34	132	205						
20	50	136	273						
29	61	56	224						
38	115	30	330						
47	16		50						
56	56		21						
62	38		43						
68	40		47	153	286	98	87	25	17
74	22		34	219	428	154	139	55	22
83	26		32	170	440	148	140	93	22
92	11		15	57	113	39	35	11	7
98	20		30	61	102	34	31	22	16
110	7		10					1	0.5

Concentrations are those in suspended particulate matter samples from two stations in the Black Sea. Compound designations are as follows: Fxn, fucoxanthin; 19'-HFxn, 19'-hexanoylfucoxanthin; Chl-*a*, chlorophyll-*a*; GBChl, bacteriochlorophyll-*e* geranyl ester; EBChl, 4-ethyl bacteriochlorophyll-*e*; PBChl, 4-*n*-propyl-bacteriochlorphyll-*e*, BBChl, 4-*i*-butyl-bacteriochlorophyll-*e*; IR, isorenieratene; β-IR, β-isorenieratene. Blank spaces denote nondetectable (<1 ng l^{-1}) pigments.

times (HPLC) with authentic standards. The fourth component (23% of the total) did not elute with any known bacteriochlorophyll-*e*. The chromatographic and spectroscopic properties of the compound initially indicated that the compound was a diastereomer or the 4-ethyl, 5-methyl homologue of bacteriochlorophyll-*e*. Diasteromers of bacteriochlorophyll-*e* have been isolated from two strains of (cultured) *Chlorobium phaeovibrioides*[11], but separation of these diastereomers by using a reverse-phase HPLC system similar to the one used here was not achieved, and it seemed unlikely that the modifications to our chromatographic analyses would have achieved such a separation. The chemical ionization (methane) mass spectrum of the pure bacteriophaeopigment that was derived from the unidentified component displayed a molecular ion at $m/z = 758$ (M+1) with major fragment ions at $m/z = 740$ (M+1−water), 622 and 604 (Fig. 3). Major fragment ions at $m/z = 622$ (macrocyle) and 604 (macrocycle-water) were also present in the mass spectrum of 4-*i*-butyl-bacteriophaeofarnesyl-*e* (derived from 4-*i*-butyl-bacteriochlorophyll-*e*). The two compounds share a common macrocycle, but differ in the esterifying alcohol. Other bacteria of chlorobiaceae are known to produce bacteriochlorophyll-*c* and -*d* with a suite of esterifying alcohols. Only the farnesyl ester of bacteriochlorophyll-*e*, however, has been reported. The mass difference of 68 AMU between the molecular ions of 4-*i*-butyl-bacteriochlorophyll-*e* and the new bacteriochlorophyll in our sample corresponds to a difference of C_5H_8 (isoprene) in the esterifying alcohol. Therefore, we tentatively identify the new bacteriochlorophyll-*e* in our samples as the geranyl ester of 4-*i*-butyl-bacteriochlorophyll-*e*. It is interesting to note that, although the ethyl, *n*-propyl, and *i*-butyl homologues of bacteriochlorophyll-*e* occurred in nearly constant proportions in all our samples (irrespective of depth or station location), only the *i*-butyl homologue of geranyl-bacteriochlorophyll-*e* was detected.

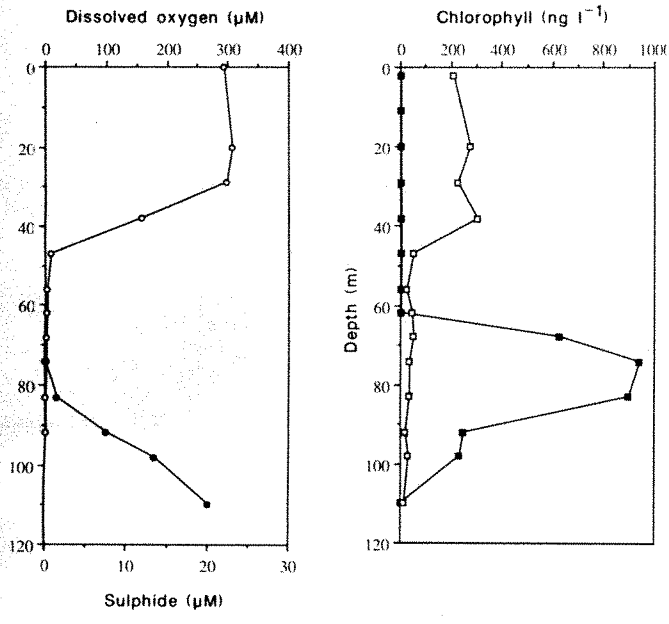

FIG. 1 The distribution of chlorophyll-*a* (□), total bacteriochlorophyll-*e* (■), dissolved oxygen (○), and hydrogen sulphide (●) at station 2 on 17 May 1988.

Concentrations of total bacteriochlorophyll-*e* reach a maximum of 214 ng l^{-1} at 100 m (station BS2-1) and 940 ng l^{-1} at 74 nm (station BS2-2) (Table 1). The lower concentration of bacteriochlorophyll-*e* at station BS2-1 is associated with a deeper chemocline and a lower ambient light intensity. From the relative distribution of pigments at the two stations, bacterial growth seems to be limited by penetration of light into the sulphide-containing deep water. A 0.1%-light-level depth of

Hashwa and Trüper[5] did not find viable photosynthetic bacteria in Black Sea water sampled in and below the chemocline, which was then at a depth of 140 m. Isolates of photosynthetic bacteria from sediment (collected at 2,000 m) were attributed to survival as chemo-organotrophs on dissolved organic matter. Our results signify that at the H_2S interface depth of 80–100 m that exists now, anaerobic photosynthesis has become a singificant process in the Black Sea carbon cycle. The shallowing of the chemocline was accompanied by the separation of the oxygenated upper layer and the shallowest appearance of H_2S by a suboxic layer ($<5\ \mu M\ O_2$) of 20–30 m (Fig. 1). Considerations of possible electron acceptors for anaerobic H_2S oxidation support the phototrophic reduction of CO_2 to organic carbon. Thus, the Black Sea supports the largest and deepest extant community of anoxygenic photosynthetic bacteria in the world, and may serve as a suitable model for studies of carbon recyling in the suboxic and anoxic seas of Cambrian and Precambian times. ☐

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1. Kriss, A. E. & Rukina, E. A. *Dokl. Akad. Nauk. SSSR* **93,** 1107–1110 (1953).
2. Sorokin, Y. I. *J. Cons. Int. Explor. Mer.* **29,** 41–53 (1964).
3. Dickman, M. & Artuz, I. *Nature* **275,** 191–195 (1978).
4. Jannasch, H. W., Trüper, H. G., and Tuttle, J. H. in *The Black Sea-Geology, Chemistry and Biology* (eds Degens, E. & Ross, D.) 419–425 (Mem. No. 20, Am. Assn. Petrol. Geol., Tulsa, Oklahoma, 1974).
5. Hashwa, F. A. & Trüper, H. G., *Helgioländer wiss. Meeresunters.* **31,** 249–253 (1978).
6. Deuser, W. G. *Deep Sea Res.* **18,** 995–1004 (1971).
7. Murray, J. W. *et al. Nature* **338,** 411–413 (1989).
8. Liaaen-Jensen, S. *Pure appl. Chem.* **57,** 649–658 (1985).
9. Wright, S. and Jeffrey, S. W. *Mar. Ecol. Prog. Ser.* **38,** 259–266 (1987).
10. Liaaen-Jensen, S. in *Marine Natural Products* Vol. 2 (ed. Scheuer, P. J.) 2–73 (Academic, New York, 1978).
11. Simpson, D. J. & Smith, K. M. *J. Am. chem. Soc.* **110,** 1753–1758 (1988).
12. Caple, M. B., Chow, H. & Strouse, C. E. *J. biol. Chem.* **253,** 6730–6737 (1978).
13. Smith, K. M., Craig, G. W., Kehres, L. A. & Pfennig, N. *J. Chrom.* **281,** 209–223 (1983).
14. Karl, D. M. & Knauer, G. A. *EOS* **69,** 1241 (1988).
15. Wirsen, C. O. and Jannasch, H. W. *EOS* **69,** 1241 (1988).
16. Liaaen-Jensen, S. *Acta chem. Scand.* **19,** 1025–1028 (1965).
17. Pfennig, N. in *The Photosynthetic Bacteria* (eds Clayton, R. K. & Sistrom, W. R.) 3–18 (Plenum, New York, 1978).
18. Brockman, H., Jr *Phil. Trans. R. Soc. Ser. B* **273,** 277–285 (1976).
19. Trüper, H. G. & Genovese, S. *Limnol. Oceanogr.* **13,** 225–232 (1968).
20. Bergstein, T., Henis, Y., and Cavari, B. Z. *Can. J. Microbiol.* **25,** 999–1007.

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Distribution of two distinct Ca^{2+}-ATPase-like proteins and their relationships to the agonist-sensitive calcium store in adrenal chromaffin cells

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MANY cellular functions are regulated by activation of cell-surface receptors that mobilize calcium from internal stores sensitive to inositol 1,4,5-trisphosphate (Ins(1,4,5)P_3) (ref. 1). The nature of these internal calcium stores and their localization in cells is not clear and has been a subject of debate. It was originally suggested[2–5] that the Ins(1,4,5)P_3-sensitive store is the endoplasmic reticulum, but a new organelle, the calciosome, identified by its possession of the calcium-binding protein, calsequestrin[6], and a Ca^{2+}-ATPase-like protein[7] of relative molecular mass 100,000 (100K), has been described as a potential Ins(1,4,5)P_3-sensitive calcium store. Direct evidence on whether the calciosome is the Ins(1,4,5)P_3-sensitive store is lacking. Using monoclonal antibodies raised against the Ca^{2+}-ATPase of skeletal muscle sarcoplasmic reticulum[8], we show that bovine adrenal chromaffin cells contain two Ca^{2+}-ATPase-like proteins with distinct subcellular distributions. A 100K Ca^{2+}-ATPase-like protein is diffusely distributed, whereas a 140K Ca^{2+}-ATPase-like protein is restricted to a region in close proximity to the nucleus. In addition, Ins(1,4,5)P_3-generating agonists result in a highly localized rise in cytosolic calcium concentration ($[Ca^{2+}]_i$) initiated in a region close to the nucleus, whereas caffeine results in a rise in $[Ca^{2+}]_i$ throughout the cytoplasm. Our results indicate that chromaffin cells possess two calcium stores with distinct Ca^{2+}-ATPases and that the organelle with the 100K Ca^{2+}-ATPase is not the Ins(1,4,5)P_3-sensitive store.

Three monoclonal antibodies raised against the Ca^{2+}-ATPase of skeletal muscle sarcoplasmic reticulum[8] were used to identify the distribution of putative calcium stores in adrenal chromaffin cells. The antibodies fortuitously had different reactivities with chromaffin cell polypeptides. In chromaffin cells (Fig. 1), monoclonal antibody 1/2H7 recognized a polypeptide of relative molecular mass 140K, whereas monoclonal Y/1F4 recognized a distinct polypeptide of relative molecular mass 100K. Monoclonal antibody B/3D6 was used as a control as it did not recognize any chromaffin cell polypeptides in immunoblotting

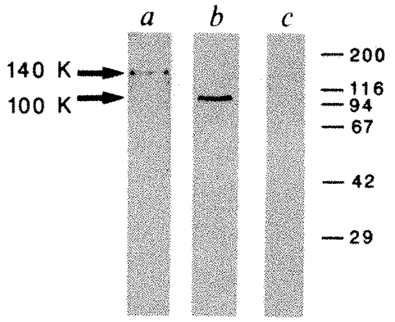

FIG. 1 Characterization of polypeptides recognized by monoclonal antibodies against skeletal muscle Ca^{2+}-ATPase in adrenal chromaffin cells. After SDS-PAGE, polypeptides recognized by the antibodies were determined by immunoblotting with *a*, 1/2H7; *b*, Y/1F4; and *c*, B/3D6. On the right are indicated the migration positions of molecular weight standards (in thousands).

METHODS. Chromaffin cells were dissociated from bovine adrenal medulla and grown in 24-well trays at a density of 7×10^5 cells per well as described previously[10]. After 24 h in culture the cells were solubilized in 150 µl of SDS-solubilization buffer[25] and polypeptides separated by SDS-PAGE on 10% slab gels. After transfer of proteins to nitrocellulose by transverse electrophoresis, the blots were stained with Ponceau S, washed with PBS medium and incubated with 3% non-fat skimmed milk in PBS for 30 min, followed by incubation for 60 min with the monoclonal antibodies diluted (1:250 for 1/2H7, 1:100 for Y/1F4 and 1:250 for B/3D6) in PBS. The blots were then incubated sequentially with anti-mouse biotin (Amersham; diluted 1:100) for 60 min and horseradish peroxidase-streptavidin (Amersham; diluted 1:500) for 30 min in 3% non-fat skimmed milk, 0.5% Tween 20 in PBS, and the reaction developed with diaminobenzidine hydrochloride/H_2O_2.

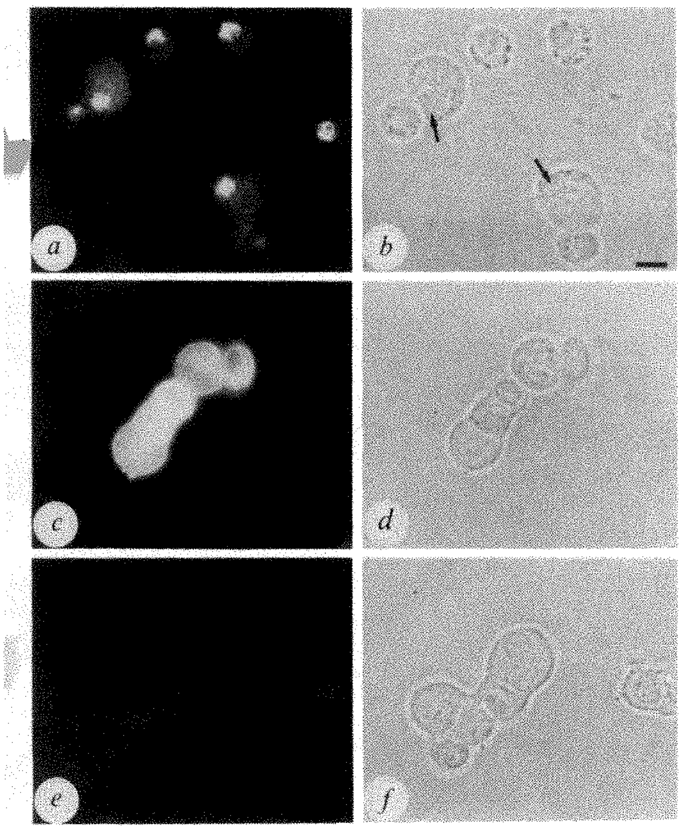

FIG. 2 Immunofluorescence localization of Ca^{2+}-ATPase-like proteins in adrenal chromaffin cells. Chromaffin cells were stained with 1/2H7 (*a, b*), Y/1F4 (*c, d*) or B/3D6 (*e, f*). Fluorescence images are shown in *a, c, e* and corresponding phase-contrast images in *b, d, f*. Antibody 1/2H7 gives a localized staining pattern restricted to a region over the nucleus of the cells. In some cases the whole of the nucleus is not stained, it being restricted to a region at the back. By contrast, Y/1F4 gives a diffuse staining pattern over the whole cell and B/3D6 does not stain the cells. Scale bar in *b*, 10 μm.

METHODS. Adrenal chromaffin cells were grown in culture overnight on glass coverslips and fixed in 4% formaldehyde. The cells were washed in PBS and incubated in 0.3% BSA, 0.1% Triton X-100 in PBS (PBT) for 30 min, followed by incubation with the monoclonal antibodies diluted in PBT (1:20 for B/3D6 and Y/1F4, and 1:250 for 1/2H7) for 60 min. The cells were then incubated sequentially with anti-mouse biotin (Amersham; 1:100) for 60 min and Texas-red streptavidin (Amersham 1:50) for 30 min.

and did not give any staining in immunofluorescence on cultured chromaffin cells (Fig. 2). The 140K protein detected by 1/2H7 was restricted in its distribution to the nuclear region of the cells. This staining of the nucleus was not always uniform but was restricted in some cases to the region facing the plasma membrane, on the side opposite the bulk of the cytoplasm (Fig. 2*a* and *b*). This staining pattern found with 1/2H7 indicates that the 140K protein is either a component of endoplasmic reticulum (ER) membranes closely associated with the nuclear envelope, or a component of the nuclear envelope itself. Using immunoblotting, we found the 140K protein to be present in a microsomal fraction of adrenal medulla, suggesting that the protein is in the ER (data not shown). By contrast, the 100K protein recognized by Y/1F4 was found to be diffusely distributed in chromaffin cells (Fig. 2*c, d*). The distribution of the 100K protein was not due to its being a component of the secretory granules (data not shown).

This variation in the distribution of the 140K and 100K proteins was found to correspond to the spatial organization of the calcium signals induced by stimuli that act through different calcium stores. First, the site of the rise in $[Ca^{2+}]_i$ in response to agonists such as methacholine, angiotensin I and angiotensin II was established in single fura-2-loaded cells by video imaging[9-11] and the nucleus was then located by staining the same cells with ethidium bromide. We had previously shown that rises in $[Ca^{2+}]_i$ in chromaffin cells in response to $Ins(1,4,5)P_3$-generating agonists, are spatially restricted and are initiated in one pole of the cells[9,10]. These rises occur even in the absence of external calcium. About 60% of cells showed a polarized rise in $[Ca^{2+}]_i$, and in these cells, challenged with methacholine ($n = 22$), angiotensin I ($n = 11$) or angiotensin II ($n = 17$), the rise in $[Ca^{2+}]_i$ originated in a region of the cell between the nucleus and the plasma membrane (Fig. 3*a*). Thus, the $Ins(1,4,5)P_3$-sensitive calcium store and the 140K Ca^{2+}-ATPase share a similar localization close to the nucleus in chromaffin cells. Recent cell fractionation studies[12] and the immunocytochemical localization of the $Ins(1,4,5)P_3$ receptor[13] provide evidence that a portion of the ER is the $Ins(1,4,5)P_3$-sensitive calcium store. Our observation that the $Ins(1,4,5)P_3$-sensitive store in chromaffin cells lies near the nucleus is consistent with the finding that the ER surrounding the nucleus of cerebellar Purkinje cells stains with an antibody against the $Ins(1,4,5)P_3$ receptor[13]. The localization of the mobilizable calcium store next to the nucleus may be important in the regulation of events within the nucleus. In the bovine adrenal chromaffin cell, a rise in $[Ca^{2+}]_i$ due to release from the internal stores is relatively ineffective in eliciting secretion[9-11] but could regulate

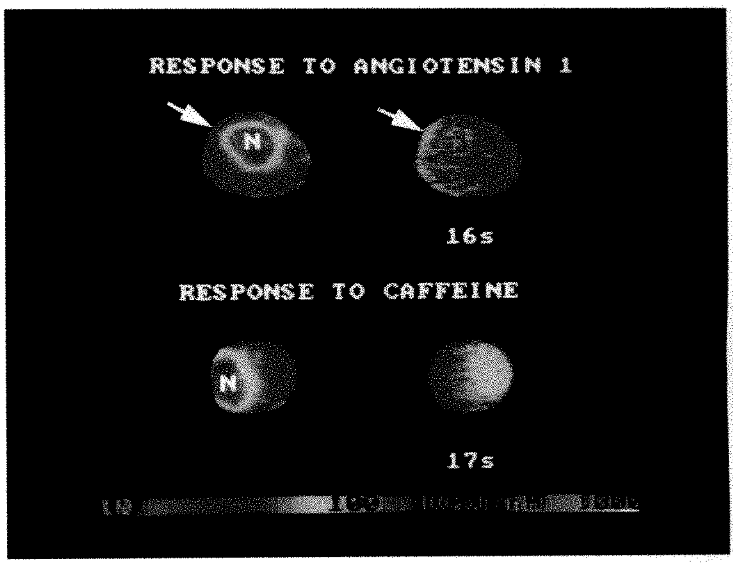

FIG. 3 Localization of the agonist-induced rise in $[Ca^{2+}]_i$ in the nucleus in chromaffin cells by video-imaging. False-colour images show on the left the location of the nucleus (N) in each cell and on the right the change in $[Ca^{2+}]_i$ due to 0.3 μM angiotensin I or 10 mM caffeine. In each case the basal $[Ca^{2+}]_i$ was subtracted from the level after stimulation so that the figures show the change in $[Ca^{2+}]_i$ from basal levels. The arrows point to the region with the highest rise in $[Ca^{2+}]_i$ (144 nM above basal; mean basal 60 nM) in response to angiotensin I. The maximum increase in $[Ca^{2+}]_i$ in response to caffeine in the cell shown was 139 nM above basal level (mean basal 64 nM).

METHODS. Imaging of $[Ca^{2+}]_i$ in single fura-2-loaded cells was carried out as described previously[9,10]. Fluorescent images were obtained by alternate excitation at 340 nm or 380 nm (40 ms at each wavelength) using an image-processing system (Imagine, Synoptics Ltd, Cambridge UK) interfaced to a DEC microvax II microcomputer. The ratio image was obtained at video rate and filtered with a time constant of 200 ms. Determination of $[Ca^{2+}]_i$ was as described by equation 5 of ref. 26. Finally, the cells were stained with 5 mg ml^{-1} ethidium bromide.

other aspects of cell function such as synthesis of biosynthetic enzymes and peptide precursors.

A different pattern of localization of $[Ca^{2+}]_i$ was seen in response to caffeine ($n = 31$), which also releases Ca^{2+} from internal stores in chromaffin cells[14]. In this case, the rise in $[Ca^{2+}]_i$ was low over the nucleus but occurred throughout the cytoplasm of the cells (Fig. 3*b*). Similar results were found in the absence of external calcium. It has recently been suggested[6] that the Ins(1,4,5)P_3-sensitive calcium store is the calciosome. This organelle in hepatocytes and pancreatic acinar cells is stained by a polyclonal antiserum against the skeletal muscle sarcoplasmic reticulum Ca^{2+}-ATPase[7]. This antiserum recognizes a 100K Ca^{2+}-ATPase-like protein in liver[15]. The calciosomes are distributed throughout the cytoplasm in many cell types, including adrenal chromaffin cells[7]. In agreement with these findings, we found that Y/1F4, which recognizes a 100K Ca^{2+}-ATPase-like protein, gives a diffuse staining pattern in chromaffin cells. As the rise in $[Ca^{2+}]_i$ in response to Ins(1,4,5)P_3-mobilizing agonists is usually spatially restricted in adrenal chromaffin cells[9,10], this indicates that the calciosomes are unlikely to be the Ins(1,4,5)P_3-sensitive calcium store. Instead, the calciosome is likely to be the caffeine-sensitive store because the rise in $[Ca^{2+}]_i$ following treatment with caffeine had a similar general distribution to that found for the 100K Ca^{2+}-ATPase-like protein.

The major finding of this study is that chromaffin cells possess two calcium stores which are spatially distinct and which differ in their sensitivity to Ins(1,4,5)P_3-mobilizing agonists, and possibly in the nature of their Ca^{2+}-ATPase. The possibility of the existence of two separate calcium stores, one sensitive to agonists that generate Ins(1,4,5)P_3 and one insensitive, has been inferred for a variety of other cell types[5,16–20]. In smooth muscle cells[17,20] and sensory neurons[18], the Ins(1,4,5)P_3-insensitive store is caffeine-sensitive. A similar situation appears to exist in chromaffin cells, where we have shown that the two stores are spatially distinct. The existence of two internal calcium stores has been invoked in one model for the mechanism of calcium oscillations[21] in response to Ins(1,4,5)P_3-generating agonists[22–24]. The ability to discriminate between these two stores in chromaffin cells may help in the dissection of the interactions and functional roles of the Ins(1,4,5)P_3-sensitive and Ins(1,4,5)P_3-insensitive stores in calcium signalling. □

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1. Berridge, M. J. *A. Rev. Biochem.* **56,** 159–193 (1987).
2. Bayerdorffer, E., Streb, H., Eckhardt, L., Haase, W. & Schulz, J. *J. Memb. Biol.* **81,** 69–82 (1984).
3. Prentki, M. *et al. Nature* **309,** 562–564 (1984).
4. Streb, H., Bayerdorffer, E., Haase, W., Irvine, R. F. & Schultz, I. *J. memb. Biol.* **81,** 241–253 (1984).
5. Biden, T. J., Wollheim, C. B. & Schlegel, W. *J. biol. Chem.* **261,** 7223–7229 (1986).
6. Volpe, P. *et al. Proc. natn. Acad. Sci. U.S.A.* **85,** 1091–1095 (1988).
7. Hashimoto, S. *et al. J. Cell Biol.* **107,** 2523–2531 (1988).
8. Colyer, J., Mata, A. M., Lee, A. G. & East, J. M. *Biochem. J.* **262,** 439–447 (1989).
9. Cheek, T. R., O'Sullivan, A. J., Moreton, R. B., Berridge, M. J. & Burgoyne, R. D. *FEBS Lett.* **247,** 429–434 (1989).
10. O'Sullivan, A. J., Cheek, T. R., Moreton, R. B., Berridge, M. J. & Burgoyne, R. D. *EMBO J.* **8,** 401–411 (1989).
11. Cheek, T. R. *J. Cell Sci.* **93,** 211–216 (1989).
12. Ghosh, T. K., Mullaney, J. M., Tarazi, F. I. & Gill, D. L. *Nature* **340,** 236–239 (1989).
13. Ross, C. A. *et al. Nature* **339,** 468–470 (1989).
14. Poisner, A. M. *Proc. Soc. exp. Biol. Med.* **142,** 103–105 (1973).
15. Damiani, E., Spamer, C., Heilmann, C., Salvatori, S. & Magreth, A. *J. biol. Chem.* **263,** 340–343 (1988).
16. Gill, D. L., Ueda, T., Chueh, S. H. & Noel, M. W. *Nature* **320,** 461–464 (1986).
17. Kanaide, H., Shogakiuchi, Y. & Nakamura, M. *FEBS Lett.* **214,** 130–134 (1987).
18. Thayer, S. A., Perney, T. M. & Miller, R. J. *J. Neurosci.* **8,** 4089–4097 (1988).
19. Busa, W. B., Ferguson, J. E., Joseph, S. K., Williamson, J. R. & Nucitelli, R. *J. Cell Biol.* **101,** 677–682 (1985).
20. Saida, K. & van Breemen, C. *Pflugers Arch.* **397,** 166–167 (1983).
21. Woods, N. M., Cuthbertson, K. S. R. & Cobbold, P. H. *Nature* **319,** 600–602 (1986).
22. Berridge, M. J. *J. Physiol., Lond.* **403,** 589–599 (1988).
23. Berridge, M. J. & Galione, A. *Fedn Proc.* **2,** 3074–3082 (1988).
24. Thomas, A. P. *J. biol. Chem.* (in the press).
25. Burgoyne, R. D. & Morgan, A. *FEBS Lett.* **245,** 122–126 (1989).
26. Grynkiewicz, G., Poenie, M. & Tsien, R. Y. *J. biol. Chem.* **260,** 3440–3450 (1985).

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Inhibition of noradrenaline release by antibodies to B-50 (GAP-43)

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PROTEIN kinase C (PKC) is believed to have a crucial role in synaptic transmitter release[1–4] and long-term potentiation[5–7]. An important substrate of PKC in the brain is the neuron-specific presynaptically localized protein B-50 (also termed GAP-43, F1, pp46 or P-57)[8–11]. B-50 has been implicated in the regulation of polyphosphoinositide metabolism[12,13] and calmodulin binding[14], and in the mechanisms of neurite outgrowth[15,16], long-term potentiation[17] and transmitter release[18]. It is still unknown, however, whether B-50 (and/or its phosphorylation) is essential to any of these processes. Here we report the results of studies in which antibodies to B-50, which interfere with B-50 phosphorylation, were introduced into rat cortical synaptosomes that were permeabilized with streptolysin-O (SL-O). We found that the release of [^{3}H]noradrenaline, induced by increasing the Ca^{2+} concentration in the buffer, is inhibited completely by the antibodies. These results provide the first demonstration of a causal relationship between the PKC substrate B-50 and the release of neurotransmitter.

Depolarization of intact rat cortical synaptosomes with 10 mM K^+ induced an efflux of noradrenaline (NA) (Fig. 1*a*, inset). This K^+-induced efflux was dependent on extracellular Ca^{2+}, confirming that in our system an influx of Ca^{2+} was the intracellular trigger for depolarization-induced neurotransmitter release. To introduce anti-B-50 IgG into synaptosomes, we used a SL-O permeation technique originally developed to study Ca^{2+}-induced vesicular histamine release from mast cells[19]. The effect of K^+ depolarization on NA release could be mimicked in SL-O-permeabilized synaptosomes by increasing the Ca^{2+} concentration in the incubation buffer from 10^{-7} to 10^{-5} M (Fig. 1*a*). To measure basal NA efflux, we used 10^{-7} M Ca^{2+} because (1) this is the resting, intracellular Ca^{2+} concentration in synaptosomes[20], and (2) a further reduction of the Ca^{2+} concentration to 10^{-8} M did not reduce NA efflux (see also Fig. 3). As in mast cells, the effect of Ca^{2+} on transmitter release could only be observed in a limited dose range of SL-O (0.6–2.0 IU ml^{-1}; Fig. 1*a*). The effect of Ca^{2+} was not due to an efflux of cytosolic NA, because Ca^{2+} did not stimulate the SL-O-induced efflux of the cytosolic marker protein, lactate dehydrogenase (Fig. 1*b*). This confirmed that the Ca^{2+}-dependent part of the SL-O-induced NA efflux was vesicular NA release. From our data we concluded that 0.8 IU ml^{-1} SL-O is the optimal concentration for permeation of the synaptosomes and for triggering the release of NA with Ca^{2+}.

We tested the effect of anti-B-50 IgG on the extent of endogenous B-50 phosphorylation in synaptosomes, measured with exogenous [γ-^{32}P]ATP as a phosphate donor. B-50 phosphorylation was only detectable after permeation of synaptosomes with SL-O (Fig. 2), because ATP does not penetrate the intact synaptosome. In control experiments in which H_2O-lysed synaptosomes were used, SL-O had no direct effect on overall kinase activity nor on B-50 phosphorylation. In the SL-O-permeabilized system, anti-B-50 IgG completely inhibited B-50 phosphorylation, whereas control IgG had no effect (Fig. 2). The inhibitory effect of anti-B-50 IgG was highly specific for the phosphorylation of B-50. These results demonstrate that the synaptosomes are sufficiently permeabilized to

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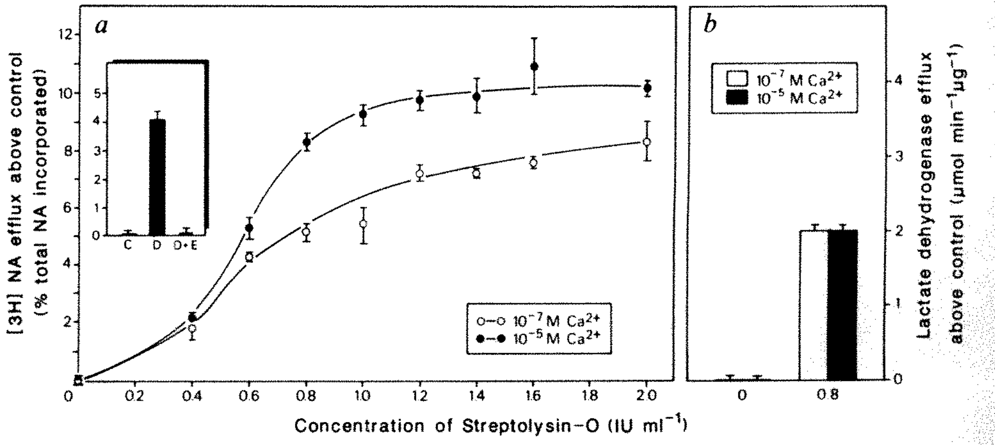

FIG. 1 *a*, Ca^{2+}-induced efflux of NA in SL-O-permeabilized synaptosomes. Synaptosomes were permeabilized with different concentrations of SL-O at 10^{-7} M (○) and 10^{-5} M (●) free Ca^{2+}. The inset shows Ca^{2+}-dependent K^+-induced release of NA in non-permeabilized synaptosomes (C, control; D, K^+-depolarized; D+E, K^+-depolarized in Ca^{2+}-free buffer). NA efflux above control is expressed as % of total NA incorporated. Control efflux (in the absence of SL-O) was 14.73% and 11.36% for Fig. 1*a* and Fig. 1*a* inset respectively. Data are expressed as mean ± s.e.m. ($n = 3$) *b*, Efflux of the cytosolic marker protein lactate dehydrogenase from permeabilized and non-permeabilized synaptosomes. Synaptosomes were treated as described below, except that lactate dehydrogenase activity instead of NA was measured in the supernatant. Control efflux (in the absence of SL-O) was 0.5 µmol min^{-1} $µg^{-1}$. Lactate dehydrogenase activity was determined according to Bergmeyer and Bernt[32].

METHODS. Rat cortical synaptosomes were isolated and labelled with NA according to De Langen *et al.*[31] using the buffer described in ref. 19, supplemented with 2 mM $CaCl_2$. They were then washed three times with buffer without $CaCl_2$ and resuspended at 2 mg protein ml^{-1}. Non-permeabilized synaptosomes (inset): synaptosomes (20 µg protein per 120 µl) were incubated for 5 min at 37 °C in buffer with 2 mM $CaCl_2$ (C), buffer containing 2mM $CaCl_2$ and 10 mM K^+ (D) or in buffer containing 10 mM K^+ and 1 mM EGTA (D + E). The samples were centrifuged for 30 s at 1,000*g*, supernatants were collected and counted in a liquid scintillation counter (Beckman 2000CA). Permeabilized synaptosomes: synaptosomes (20 µg protein per 120 µl) were incubated for 5 min at 37 °C in buffer containing various concentrations of SL-O (Wellcome, UK), 5 mM ATP, 2 mM $MgCl_2$ and Ca^{2+}-EGTA buffers with 10^{-7} M or 10^{-5} M free Ca^{2+} (ref. 19). Samples were then processed as described above.

introduce anti-B-50 IgG and that these antibodies specifically inhibit B-50 phosphorylation.

Next we incubated permeabilized synaptosomes with anti-B-50 IgG and applied the Ca^{2+} trigger, and measured NA release. Ca^{2+}-induced release of NA was inhibited by the anti-B-50 IgG in a concentration-dependent way (Fig. 3), whereas control IgG and heat-inactivated anti-B-50 IgG were ineffective at such inhibition. Neither control IgG nor anti-B-50 IgG affected NA efflux at 10^{-8} and 10^{-7} M Ca^{2+}, showing that the antibodies do not influence the Ca^{2+}-independent efflux.

We conclude that B-50 plays a crucial part in the molecular

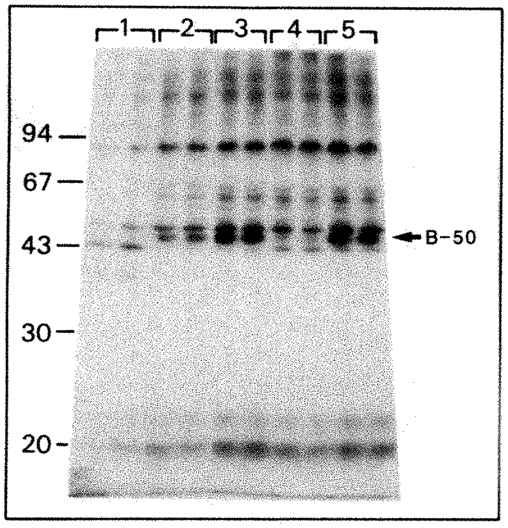

FIG. 2 Autoradiogram showing the effects of anti-B-50 IgG on endogenous protein phosphorylation in SL-O-permeabilized synaptosomes. Synaptosomes were treated with 0 (lanes 1), 0.4 (lanes 2), and 0.8 (lanes 3) IU ml^{-1} SL-O before phosphorylation with [γ-^{32}P]ATP. The effect of anti-B-50 IgG (lanes 4) or control IgG (lanes 5) was tested in synaptosomes permeabilized with 0.8 IU ml^{-1} SL-O. Position of relative molecular mass markers ($\times 10^3$) shown on left.

METHODS. Synaptosomes (10 µg protein) were permeabilized with SL-O for 5 min at 37 °C in 30 µl PIPES buffer containing 10^{-5} M Ca^{2+} without ATP. Phosphorylation was started by adding 2 µCi [γ-^{32}P]ATP (specific activity 3,000 Ci $mmol^{-1}$; Amersham, UK) and carrier ATP to a final concentration of 7.5 µM. After 15 s, phosphorylation was stopped and analysed as described[10]. IgG (10 µg per assay) were added simultaneously with SL-O. Anti-B-50 IgG was isolated from B-50 antiserum raised in rabbits as described previously[33] by affinity chromatography on a B-50 column[33]. Isolated anti-B-50 IgG was extensively dialysed against 1,000 volumes of double distilled water, freeze dried and stored dry at −80 °C. On western blots loaded with total synaptosomal proteins, we confirmed the IgG to be specific for B-50. The amount of IgG was determined according to Lowry *et al.*[34] with total rabbit IgG (Miles, UK) as reference. Total rabbit IgG was also used as control IgG.

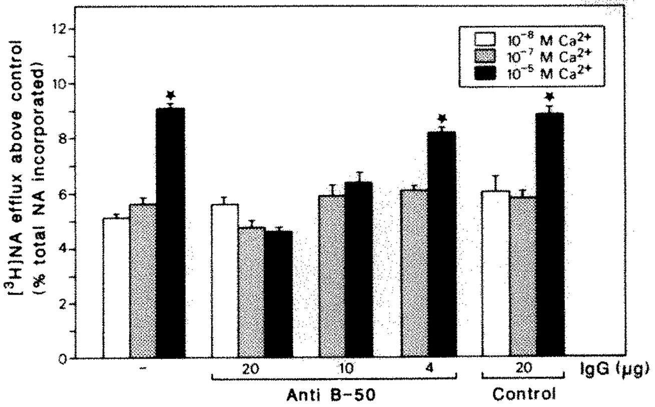

FIG. 3 Effect of anti-B-50 IgG on Ca^{2+}-induced release of NA from SL-O-permeabilized synaptosomes. Synaptosomes were permeabilized with 0.8 IU ml^{-1} SL-O and incubated without IgG (−), with different concentrations of anti-B-50 IgG (anti-B-50), or with control IgG (control) as described in Fig. 2 legend. Ca^{2+}-induced NA release was measured as described in Fig. 1 legend (open bars, 10^{-8} M Ca^{2+}; dotted bars, 10^{-7} M Ca^{2+}; filled bars, 10^{-5} M Ca^{2+}). Neither anti-B-50 nor control IgG (20 µg per assay) affected NA release in non-permeabilized synaptosomes. Data are expressed as mean ± s.e.m. (three independent experiments). Identical results were obtained in a separate experiment using a different batch of anti-B-50 IgG. Data were analysed by one-way analysis of variance (ANOVA) with a supplemental Newman-Keuls test. Asterisk denotes group significantly different ($P < 0.05$) from all groups treated with 10^{-7} or 10^{-8} M Ca^{2+}.

mechanism of NA release from rat cerebral-cortex synaptosomes. Because immunocytochemical studies have shown that B-50 is found in synapses throughout the brain[21,22], B-50 could be more generally involved in transmitter release. Three lines of evidence suggest that the phosphorylation of B-50 by PKC is essential for stimulus-secretion coupling during transmitter release: (1) phorbol esters that directly activate PKC enhance the release of a variety of neurotransmitters[1-4]; (2) by using an antibody-independent approach, we have previously shown that depolarization-induced neurotransmitter release from non-permeabilized synaptosomes and hippocampal slices is closely correlated with a PKC-mediated increase in B-50 phosphorylation[18,23]; and (3) here we have shown that anti-B-50 IgG inhibits B-50 phosphorylation as well as Ca^{2+}-dependent transmitter release. If B-50 phosphorylation by PKC is indeed involved in the mechanism of transmitter release, then a long-term increase in PKC-mediated B-50 phosphorylation[7,17] could be one of the mechanisms underlying the increase in the release of glutamate that occurs during long-term potentiation[5-7,24,25].

In view of the localization of B-50 at the inner leaflet of the plasma membrane[21,26], we suggest that B-50 is involved in the regulation of vesicle fusion with the plasma membrane, a process in which the vesicle-associated protein synapsin I (a substrate of calmodulin-dependent kinases) has also been implicated[27,28]. But the difference in the localization of phosphorylating enzymes of these two proteins indicates that they have distinct roles in the transmitter release process. It may be that the regulatory role of B-50 in vesicle fusion is not limited to transmitter release, but extends to membrane-fusion processes during neurite outgrowth[29,30]. It remains to be investigated to what extent calmodulin binding[14] and modulation of phosphatidyl inositol 4-phosphate kinate activity[12,13]—putative properties of B-50—are also involved in controlling neurotransmitter release. ☐

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1. Allgaier, C., Von Kügelgen, O. & Hertting, G. *Eur. J. Pharmacol.* **129,** 389-392 (1986).
2. Malenka, R. C., Ayoub, G. S. & Nicoll, R. A. *Brain Res.* **403,** 198-203 (1987).
3. Nichols, R. A., Haycock, J. W., Wang, J. K. T. & Greengard, P. *J. Neurochem.* **48,** 615-621 (1987).
4. Kaczmarek, L. K. *Trends Neurosci.* **10,** 30-34 (1987).
5. Malenka, R. C., Madison, D. V. & Nicoll, R. A. *Nature* **321,** 175-177 (1986).
6. Malinow, R., Madison, D. V. & Tsien, R. W. *Nature* **335,** 820-824 (1988).
7. Akers, R. F., Lovinger, D. M., Colley, P. A., Linden, D. J. & Routtenberg, A. *Science* **231,** 587-589 (1986).
8. Zwiers, H., Schotman, P. & Gispen, W. H. *J. Neurochem.* **34,** 1689-1699 (1980).
9. Aloyo, V. J., Zwiers, H. & Gispen, W. H. *J. Neurochem.* **41,** 649-653 (1983).
10. De Graan, P. N. E., Dekker, L. V., Oestreicher, A. B., Van der Voorn, L. & Gispen, W. H. *J. Neurochem.* **52,** 17-23 (1989).
11. Skene, J. H. P. *A. Rev. Neurosci.* **12,** 127-156 (1989).
12. Jolles, J. *et al. Nature* **286,** 623-625 (1980).
13. Gispen, W. H., Van Dongen, C. J., De Graan, P. N. E., Oestreicher, A. B. & Zwiers, H. in *Inositol and Phosphoinositides* (eds Blaesdale, J. E., Hauser, G. & Eichberg, J. 399-413 (Humana, Clifton, New Jersey 1985).
14. Andreasen, T. J., Luetje, C. W., Heideman, W. & Storm, D. R. *Biochemistry* **22,** 4615-4618 (1983).
15. Skene, J. H. P. & Willard, M. *J. Cell Biol.* **89,** 86-95 (1981).
16. Goslin, K., Schreyer, D. J., Skene, J. H. P. & Banker, G. *Nature* **336,** 672-674 (1988).
17. Lovinger, D. M. *et al. Brain Res.* **343,** 137-143 (1985).
18. Dekker, L. V., De Graan, P. N. E., Versteeg, D. H. G., Oestreicher, A. B. & Gispen, W. H. *J. Neurochem.* **52,** 24-30 (1989).
19. Howell, T. W. & Gomperts, B. D. *Biochim. biophys. Acta* **927,** 177-183 (1987).
20. Verhage, M., Besselsen, E., Lopes Da Silva, F. H. & Ghijsen, W. E. J. M. *J. Neurochem.* **51,** 1667-1674 (1988).
21. Gispen, W. H., Leunissen, J. L. M., Oestreicher, A. B., Verkleij, A. J. & Zwiers, H. *Brain Res.* **328,** 381-385 (1985).
22. Benowitz, L. I., Apostolides, P. J., Perrone-Bizzozero, N., Finklestein, S. P. & Zwiers, H. *J. Neurosci.* **8,** 339-352 (1988).
23. Schrama, L. H. *et al. Soc. Neurosci.* Abstr. **14,** 197.15 (1988).
24. Dolphin, A. C., Errington, M. L. & Bliss, T. V. P. *Nature* **297,** 496-498 (1989).
25. Aniksztejn, L., Roisin, M. P., Amsellam, R. & Ben-Ari, Y. *Neuroscience* **28,** 387-392 (1989).
26. Skene, J. H. P. & Virág, I. *J. Cell Biol.* **108,** 613-624 (1989).
27. Bähler, M. & Greengard, P. *Nature* **326,** 704-707 (1987).
28. Hirokawa, N., Sobue, K., Kanda, K., Harada, A. & Yorifuji, H. *J. Cell Biol.* **108,** 111-126 (1989).
29. van Hooff, C. O. M. *et al. J. Cell Biol.* **108,** 1115-1125 (1989).
30. Zuber, M. X., Goodman, D. W., Karns, L. R. & Fishman, M. C. *Science* **244,** 1193-1195 (1989).
31. De Langen, C. D. J., Hogenboom, F. & Mulder, A. H. *Eur. J. Pharmacol.* **60,** 79-89 (1979).
32. Bergmeyer, H. U. & Bernt, E. in *Methoden der Enzymatischen Analyse* (ed. Bergmeyer, H. U.) 533-538 (Verlag Chemie, Weinheim, 1970).
33. Oestreicher, A. B., van Dongen, C. J., Zwiers, H. & Gispen, W. H. *J. Neurochem.* **41,** 331-340 (1983).
34. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. *J. biol. Chem.* **193,** 265-275 (1951).

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Production of antibodies in transgenic plants

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COMPLEMENTARY DNAs derived from a mouse hybridoma messenger RNA were used to transform tobacco leaf segments followed by regeneration of mature plants. Plants expressing single gamma or kappa immunoglobulin chains were crossed to yield progeny in which both chains were expressed simultaneously. A functional antibody accumulated to 1.3% of total leaf protein in plants expressing full-length cDNAs containing leader sequences. Specific binding of the antigen recognized by these antibodies was similar to the hybridoma-derived antibody. Transformants having γ- or κ-chain cDNAs without leader sequences gave poor expression of the proteins. The increased abundance of both γ- and κ-chains in transformants expressing assembled gamma-kappa complexes was not reflected in increased mRNA levels. The results demonstrate that production of immunoglobulins and assembly of functional antibodies occurs very efficiently in tobacco. Assembly of subunits by sexual cross might be a generally applicable method for expression of heterologous multimers in plants.

The source of immunoglobulin mRNAs was a hybridoma cell line expressing a catalytic IgG_1 antibody (6D4) which binds a low molecular weight phosphonate ester (P3) and catalyses the hydrolysis of certain carboxylic esters. Constructs used for immunoglobulin expression in plants consisted of coding-length cDNAs of the 6D4 γ- or κ-chain with or without their leader sequences. These cDNAs were modified to contain terminal *Eco*R1 restriction enzyme digestion sites and were ligated into the constitutive plant expression vector pMON530 (ref. 2) to form pHi101 (kappa, no leader), pHi102 (kappa, leader), pHi201 (gamma, no leader) and pHi202 (gamma, leader). We transformed tobacco plants using Agrobacterium containing each of these four plasmids[3] and screened leaf extracts from regenerated transformants for the presence of immunoglobulin heavy or light chains by enzyme-linked immunosorbent assay (ELISA)[4]. Transformants expressing individual immunoglobulin chains were then sexually crossed to produce progeny expressing both chains. The results of the ELISA revealed high levels of kappa and gamma chains accumulating in individual plants containing DNA from both pHi102 and pHi202 (Table 1; Fig. 2*a*). We verified the expression of both heavy and light chains by western blotting (Fig. 1). From the ELISAs, we judged that virtually all the γ- and κ-chains in these plants were assembled into gamma-kappa complexes (Table 1). Western blots provided additional evidence for assembled antibodies in that, under non-reducing conditions, most of the immunoreactive γ- and κ-chains aggregated at a high molecular weight (Fig. 1).

The binding specificity of the assembled gamma-kappa complexes was studied in ELISAs in which a P3-bovine serum albumin conjugate was used as antigen. The antigen binding by antibody derived from plants was equivalent to antigen binding by the 6D4 hybridoma antibody. Incubation of plant extracts or the purified 6D4 antibody with 50 $\mu mol\ l^{-1}$ P3 for 3 h at 25 °C before addition to the ELISA eliminated antibody binding to the P3-BSA conjugate, demonstrating that binding was specific for the P3 hapten. Half-maximal inhibition occurred with 10 $\mu mol\ l^{-1}$ free P3 for both hybridoma and plant-derived antibodies.

Transformants derived from the leaderless constructs pHi101 and pHi201 contained very low levels of κ- and γ-chains respectively, but Southern and northern blots (Fig. 2) demonstrated the presence of transforming DNA and immunoglobulin transcripts. None of the plants expressing leaderless immunoglobulin chains contained assembled gamma-kappa complexes (Table 1).

The increased recovery of immunoglobulin epitopes from transformants expressing full-length cDNAs was not reflected in increased mRNA transcript levels. Northern blots (Fig. 2*b*) comparing pHi201 and pHi202 transformants, for example, revealed nearly equivalent levels of heavy-chain transcripts, although ELISAs indicated a 40-fold increase in accumulation of heavy-chain protein in the pHi202 transformant. Likewise, immunoglobulin mRNA levels in a plant producing large amounts of assembled antibodies were not significantly different from the parental plants that accumulated low levels of immunoglobulin chains (Fig. 2*b*).

Our results show that individual cDNAs for immunoglobulin κ- and γ-chains can be efficiently expressed in tobacco to form functional antibodies. Assembly of immunoglobulin chains by sexual cross in plants represents a useful alternative to the expression by a single vector of both gamma and kappa cDNAs as in yeast or bacteria[5-7], or double transformation with vectors containing individual cDNAs[8,9]. Potentially, this method is applicable to the assembly of oligomers other than antibodies. The characterization of antibodies produced in plants (glycosylation, processing of leader sequences, cytolocalization and turnover) will be described in a later paper.

In B lymphocytes, immunoglobulin processing and assembly occurs in the endoplasmic reticulum/Golgi in a process that may be promoted by heavy-chain binding proteins present in the endoplasmic reticulum[10,11]. Plant cells may also have a system for multimer assembly in their endoplasmic reticulum/Golgi that can recognize immunoglobulin chains. Alternatively, assembly may occur spontaneously, given sufficient levels of each chain in the appropriate cellular compartment. Our results demonstrate that plants require a signal sequence for efficient assembly of γ- and κ-subunits. The presence of the mouse leader sequence clearly augments the accumulation of individual chains. This might be the result of an enhanced translation of the immunoglobulin messengers or an increased stability of each protein as a result of subcellular sequestering or secretion. The yield of each chain is increased in plants expressing both gamma and kappa, indicating that assembly of the gamma-kappa complex might enhance stability.

TABLE 1 Expression and assembly of immunoglobulin gamma and kappa chains in tobacco

Accumulation of γ- or κ-chains in transformed plants*

γNL	**γL**	**γL(κL)**	**γNL(κNL)**
30 ± 16 (60)	1,412 ± 270 (2,400)	3,330 ± 2,000 (12,800)	32 ± 26 (60)
κNL	**κL**	**κL(γL)**	**κNL(γNL)**
1.4 ± 1.2 (3.5)	56 ± 5 (80)	3,700 ± 2,300 (12,800)	6.5 ± 5 (20)

Distribution and assembly in crosses†

	γ only	**κ only**	**γκ**	**Null**
κNL × γNL	4	6	3 (0% assembly)	5
κL × γL	3	10	11 (95 ± 16% assembly)	4

* Accumulation of individual gamma and kappa chains (in ng per mg total protein) was estimated by ELISA[4]. Microtitre wells were coated with a goat anti-mouse heavy or light chain-specific IgG (Fisher) in 150 mM NaCl, 20 mM Tris-HCl, pH 8.0 (TBS), followed by blocking with 5% non-fat dry milk in TBS. Plant leaves were homogenized in a mortar and pestle at 4 °C after removal of the midvein. To the supernatant a quarter volume of 5 × TBS was added, and 50 μl of 1 in 2 serial dilutions were added to each microtitre well. After 18 h at 4 °C, microtitre wells were washed with distilled water at room temperature. Bound γ- or κ-chains were reacted with goat anti-mouse heavy or light chain-specific antibodies conjugated to horseradish peroxidase for 2 h at 37 °C in TBS, and detected according to the manufacturer's instructions. Control microtitre wells contained extracts from plants transformed with pMON530 vector. Values given as mean (± s.d.) are derived from at least two determinations per plant and do not include transformants producing no detectable γ- or κ-chain. At least nine plants were assayed in each category. All values are given as ng per mg of total protein in the extract and are derived from the quantity of purified 6D4 antibody required to given an equivalent colour development in ELISA. Total protein in the extract was determined by the Bio-Rad Coomassie assay. Complementary DNAs containing no leader sequences are referred to as γNL and κNL; γL and κL refer to cDNAs with leader sequences; γ(κ) refers to gamma chains in a plant that also expresses κ-chains, and vice versa. Numbers in parentheses are values for plants with the highest levels of accumulation.

† The number of plants expressing γ- or κ-chains among the progeny of a sexual cross. The ELISA for assembly used horseradish peroxidase-conjugated anti-κ-chain-specific antibodies to detect antigen bound to microtitre wells coated with unlabelled anti-γ-chain-specific antibodies, and vice versa. Values derived from these assays were used to calculate the per cent of assembly by comparison with the purified 6D4 antibody. This was determined at least three times for each γκ plant. The per cent assembly is expressed in parentheses as the mean ± s.d.

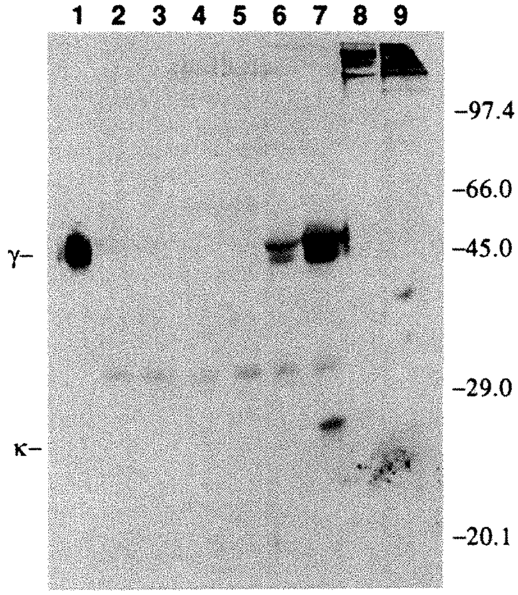

FIG. 1 Western blot of leaf proteins from transgenic tobacco plants expressing immunoglobulin chains. Leaf segments (1 g) from mature plants were homogenized in a mortar and pestle with 1 ml 0.05 M Tris-HCl, pH 7.5, 1 mM phenylmethanesulphonyl fluoride. Extracts were boiled in 4 M urea, 1% SDS, with or without 2 mM dithiothreitol (DTT) as indicated, for 3 min. SDS-PAGE in 10% acrylamide[16] and blotting of the proteins to nitrocellulose[17] were performed as described. Blots were preincubated for 6 h at 4 °C in 20 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.01% Tween 20 (TBST) containing 5% BSA, and 0.5% non-fat dried milk before the addition of antibodies. A biotinylated goat anti-mouse whole IgG antibody (Cappel), diluted 1:500 in TBST was used to probe the blots at 4 °C for 24 h. A variety of commercially available antibodies (anti-mouse IgGs) were used in other experiments with similar results. Antibody binding was visualized after binding of streptavidin-conjugated alkaline phosphatase (25 °C, for 2 h) by incubation in 300 μg ml^{-1} nitroblue tetrazolium and 150 μg ml^{-1} 5-bromo-4-chloro-3-indolyl phosphate. In lanes 1–7, 40 μl of each extract containing DTT; lanes 8 and 9, 40 μl extract without DTT. Lane 1, 100 ng purified antibody from the 6D4 hybridoma; lane 2, 15 μg wild-type plant-extract protein; lane 3, 15 μg protein from a plant transformed with truncated κ-chain cDNA (pHi101) containing no leader sequence; lane 4, 15 μg from plant transformed with truncated γ-chain cDNA (pHi201); lane 5, 15 μg from a full-length kappa cDNA transformant (pHi102); lane 6, 15 μg from a full-length γ-chain cDNA transformant (pHi202); lane 7, 15 μg from an F1 plant derived from the cross between a kappa and a gamma producer; lane 8, 100 ng 6D4 antibody (no DTT); lane 9, same as lane 7, except no DTT. Gamma and kappa on the left refer to the positions of the 6D4 heavy and light chains; positions of molecular weight (given in thousands) markers are shown on the right. By ELISA, extracts in lanes 3–5 contained very low levels of κ- or γ-chains (<0.008% of total protein, Table 1), whereas extracts in lanes 6, 7 and 9 contained 0.24, 1.3 and 1.3% immunoglobulin respectively.

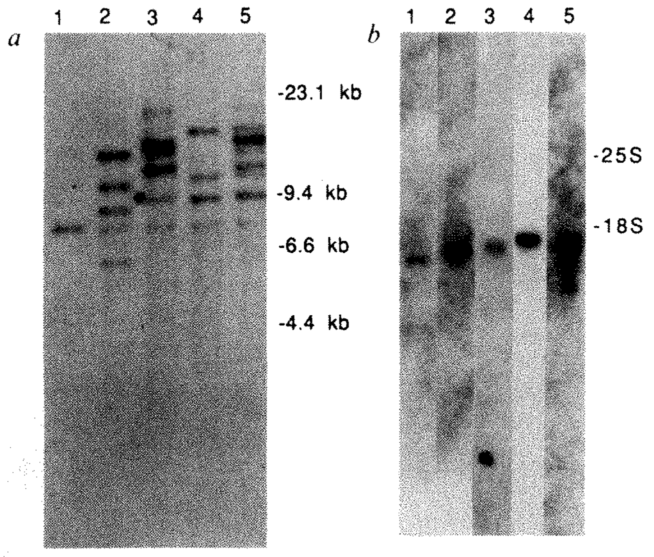

FIG. 2 Southern and northern blots of leaf DNA and RNA from transgenic plants expressing immunoglobulin cDNAs. *a*, DNA was extracted from 1 g mature leaf tissue after freezing the fresh segments in liquid N_2. Homogenization into urea mix[18] was in a mortar and pestle. The homogenate was extracted with phenol:$CHCl_3$ (1:1) and the nucleic acids precipitated by addition of one volume of isopropyl alcohol. The resuspended DNA (20 μg each) was cut with *Hin*dIII and Southern-blotted as described[19]. The probe used for both Southern and northern blots was ^{32}P-labelled pMON530 plasmid[2] containing either a kappa cDNA or a gamma cDNA. Both cDNAs were used in the hybridization shown. Lane 1, DNA from a transformant expressing a light-chain cDNA without a leader sequence (pHi101); lane 2, DNA from a heavy-chain cDNA transformant, no leader (pHi201); lane 3, DNA from a transformant expressing full-length light chain with leader (pHi102); lane 4, DNA from a transformant expressing heavy chain with leader (pHi202); lane 5, DNA from an F_1 plant derived from a cross between plants expressing full-length gamma or kappa cDNAs (pHi102 × pHi202). *b*, Extraction of RNA from 1 g fresh leaf tissue was by homogenization in 10 ml 0.1 M Tris-HCl, pH 9.0, and 10 ml phenol saturated with this buffer, using a Polytron at high speed. Nucleic acids were precipitated by addition of one tenth volume 3.0 M sodium acetate, pH 5.0, and 1.5 volumes isopropyl alcohol. Resuspended RNA was electrophoresed in gels containing formaldehyde and northern blotted onto nylon membranes (Amersham) as described[19]. Lane 1, RNA from a transformant expressing a light-chain cDNA without a leader sequence (pHi101); lane 2, RNA from a heavy-chain cDNA transformant, no leader (pHi201); lane 3, RNA from a transformant expressing full-length light chain with leader (pHi102); lane 4, RNA from a transformant expressing heavy chain with leader (pHi202); lane 5, RNA from an F_1 plant derived from a cross between plant expressing full-length gamma or kappa cDNAs (pHi102 × pHi202). Total RNA (20 μg) was loaded in each lane. Lanes from separate hybridizations were aligned with respect to the 18S (1,900 base pairs) and 25S (3,700 base pairs) ribosomal RNA bands on the blots, detected by methylene-blue staining.

Expression of functional antibodies from transcripts that do not contain signal sequences may require modifications to yield alternative antigen-binding structures (such as single-chain antigen-binding proteins[6,7]) that do not need to be assembled. Thus binding of constituents of metabolic pathways involved in morphogenesis, stress responses or plant-pathogen interactions could be used to further our understanding of these processes in a way analogous to the blocking by microinjected antibodies of specific protein functions in mammalian cells[12,13].

As large macromolecules such as protein multimers do not pass through plant cell walls[14,15], the binding by antibodies of small organic molecules (toxins, herbicides, plant hormones, organic chelates, for example) that are permeable to the cell wall might result in the net uptake and retention of these molecules in the plant. Accumulation of functioning antibodies may provide new options for the recovery of an array of environmental contaminants, as well as other biologically significant organics. Catalytic processing of small molecules within cell wall boundaries by antibodies may also become a generalized strategy for the elimination or modification of permeable organic compounds and could introduce new catalytic properties into existing metabolic pathways. ☐

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1. Tramontano, A., Janda, K. D. & Lerner, R. A. *Science* **234,** 1566-1570 (1987).
2. Rogers, S. G., Klee, H. J., Horsch, R. B. & Fraley, R. T. *Meth. Enzym.* **153,** 253-277 (1987).
3. Horsch, R. B. *et al. Science* **227,** 1229-1231 (1985).
4. Engvall, E. & Perlmann, P. *J. Immun.* **109,** 129-135 (1972).
5. Carlson, J. R. *Mol. cell. Biol.* **8,** 2638-2646 (1985).
6. Huston, J. S. *et al. Proc. natn. Acad. Sci. U.S.A.* **85,** 5879-5883 (1988).
7. Bird, R. E. *et al. Science* **242,** 423-426 (1988).
8. Wood, C. R. *et al. Nature* **314,** 446-449 (1985).
9. Horwitz, A. H., Chang, C. P., Better, M., Hellstrom, K. E. & Robinson, R. R. *Proc. natn. Acad. Sci. U.S.A.* **85,** 8678-8682 (1988).
10. Tartakoff, A. & Vassali, P. *J. Cell Biol.* **83,** 284-299 (1979).
11. Munro, S. & Pelham, H. R. B. *Cell* **46,** 291-300 (1986).
12. Lamb, N. J. C. *et al. J. Cell Biol.* **106,** 1955-1971 (1988).
13. Riabowol, K. T., Vosatka, R. J., Ziff, E. B., Lamb, N. J. & Feramisco, J. R. *Mol. cell. Biol.* **8,** 1670-1676 (1988).
14. Milburn, J. A. *Water Flow in Plants* Longman, (London, 1979).
15. Carpita, N., Sabularse, D., Montezinos, D. & Delmer, D. P. *Science* **205,** 1144-1147 (1979).
16. Chua, N-H. *Meth. Enzym.* **69,** 434-446 (1980).
17. Harlow, E. & Lane, D. *Antibodies: A Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1988).
18. Shure, M., Wessler, S. & Fedoroff, N. *Cell* **35,** 225-233 (1983).
19. Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1982).

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Leu-8/TQ1 is the human equivalent of the Mel-14 lymph node homing receptor

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THE human pan-leukocyte antigen Leu-8 has attracted wide interest because its presence or absence identifies suppressor-inducer and helper-inducer $CD4^+$ T-lymphocyte subsets respectively. We report here that Leu-8 is the human homologue of the mouse Mel-14 homing receptor, a molecule that promotes the initial adhesion of blood-borne lymphocytes to the specialized post-capillary endothelium of peripheral lymph nodes. We also show that Leu-8 can adopt both conventional and phospholipid anchored forms, a finding that may have relevance in the context of antigen shedding following activation or homing. The assignment of lymphocytes to different functional classes based on lymph node homing potential may represent a more general association between lymphocyte function and tissue distribution.

Two complementary DNA clones encoding Leu-8 determinants were isolated from a human T-cell library by methods previously described[1,2]. DNA sequence analysis showed that the longer insert of the two contains 2,350 residues, whereas the shorter lacks 436 internal residues but is otherwise identical (Fig. 1). The predicted polypeptide sequences were found to diverge at their C-termini.

The protein encoded by the larger insert bears a strongly hydrophobic putative membrane spanning domain near its C terminus, followed by several positively charged residues resembling a cytoplasmic anchor sequence. The protein is closely related to the recently described murine Mel-14 homing receptor[3,4] (Fig. 1) and the corresponding cDNA sequence shares

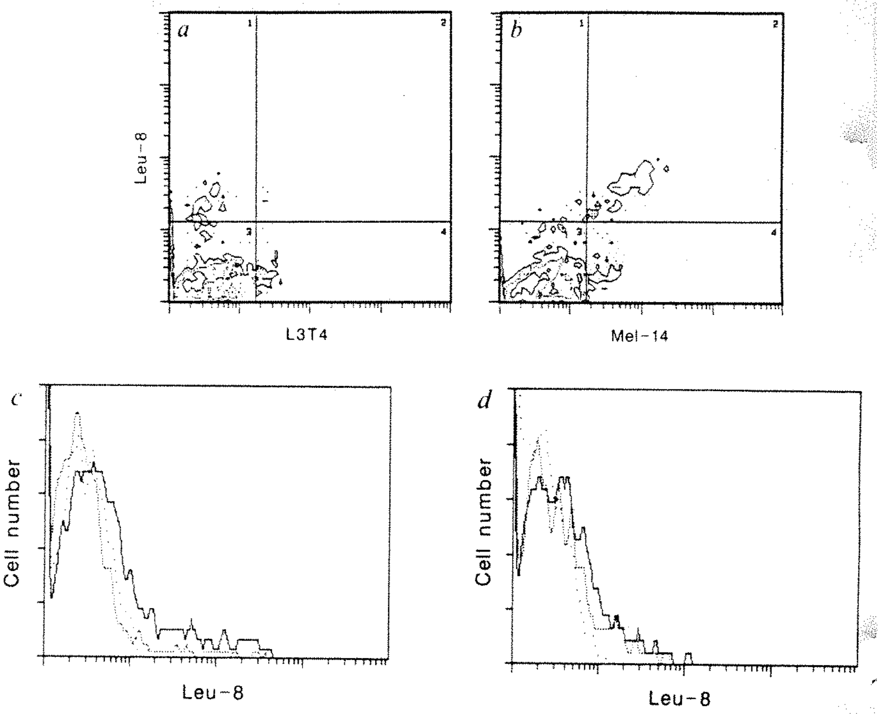


FIG. 2 Flow cytometric analysis of COS cells transfected with Leu-8 cDNA clones. *a, b,* Two-colour analysis with a phycoerythrin-conjugated anti-Leu-8 antibody (Leu-8 PE) and *a,* rat hybridoma L3T4 (control) ascites followed by fluorescein conjugated goat anti-rat IgG (fluorescein isothiocyanate (FITC) goat anti-rat IgG) or *b,* rat hybridoma Mel-14 ascites followed by FITC goat anti-rat IgG. *c, d,* Sensitivity of Leu-8 antigen to release by phosphatidylinositol-specific phospholipase C. COS cells expressing the shorter Leu-8 cDNA clone, *c,* or the longer clone *d,* were incubated with anti-Leu-8 FITC after incubation with phosphatidylinositol-specific phospholipase C or buffer alone at 37 °C for 1 h. In *c* and *d,* the histogram of CD16 (control) transfected COS cells stained with labelled anti-Leu-8 is represented by a sparsely dotted line, the histogram of Leu-8 transfected cells stained with labelled anti-Leu-8 is represented by a solid line, and the histogram of Leu-8 transfected cells treated with phospholipase C and similarly stained is represented by a broken line.

METHODS. For two-colour analysis, COS cells were trypsinized 12–24 h after DEAE-Dextran transfection and passaged in a ratio of 1:2. Post-transfection (60–72h), the cells were collected by incubation in PBS medium plus 0.5 mM EDTA (PBSE), collected by centrifugation, and resuspended in RPMI medium containing 10% human plasma. L3T4 or Mel-14 ascites was added at 1:50 dilution, and the cells were incubated on ice for 30–40 min, and centrifuged through PBS, 2% Ficoll, 1 mg ml^{-1} BSA, 0.04% sodium azide (PFBA). The cells were resuspended in PBSE with 20% skim milk containing affinity purfied FITC conjugated goat anti-rat IgG (Chemicon) at 50 μg ml^{-1}. After 20 min on ice, the cells were diluted with PBS plus 0.5 mM EDTA, and centrifuged through a two-step gradient of PFBA (0.25 ml) over PBS with 4% formaldehyde (2.5 ml). The cells were allowed to fix for 15 min, then the supernatant was aspirated, and the pellet and cells adhering to the walls of the tube were resuspended in IMD medium containing 0.02% Nonidet P-40 detergent, and recentrifuged. Anti-Leu-8 PE was added, the cells were incubated on ice for 30 min and then centrifuged through PBS, with 4% formaldehyde and 0.05% Tween-20, and stored for cytometry. For single-colour analysis, the primary antibody was added to the cells in PBS with 0.02% NaN$_3$ and 5% fetal bovine serum and incubated for 1 h on ice. Cells were washed as for the two-colour analyses. Incubation with phosphatidylinositol-specific phospholipase C was for 1 h at 37 °C in 2.6% glycerol and PBS at a ratio of 9:1.

single copy gene (Fig. 3). RNA blot hybridization revealed a major transcript of ~2.4 kilobases (kb) present in peripheral blood mononuclear cells, tonsillar B cells, and several lymphocytic cell lines; and a minor transcript of 2.0 kb, present in peripheral blood mononuclear cells, and the Jurkat and HSB-2 leukaemic T-cell lines (Fig. 3, and data not shown). Reduced expression of the RNA was detected in peripheral blood lymphocytes activated with phorbol ester plus either phytohaemagglutinin or concanavalin A, whereas treatment with phorbol ester alone induced transcript expression (Fig. 3). Tonsillar B cells activated by anti-IgM antibody, phorbol diester, anti-CD20 monoclonal antibody, G28-8 monoclonal antibody, or gamma interferon showed little change in transcript abundance (Fig. 3).

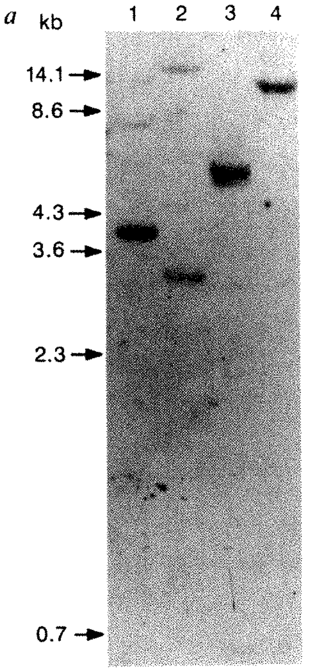
Western blot analysis allowed at least seven species of similar molecular weight to be visualized from extracts of Leu-8-transfected COS cells (Fig. 3). Extracts prepared from the human T-cell leukaemia line Jurkat, which expresses Leu-8 transcripts and surface antigen (data not shown), showed at least two closely spaced bands with a relative molecular mass of ~76,000 (76 K), the size of the largest forms seen in COS cells.

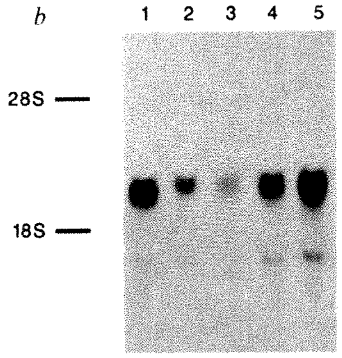
Twelve hours after activation by phorbol ester plus phytohaemagglutinin, Leu-8 expression by peripheral blood lymphocytes is diminished, whereas Leu-8 transcripts are more prevalent (Fig. 4). At 24 h post-treatment, both surface and transcript expression are substantially reduced (Fig. 4). Thus, surface Leu-8 is lost more rapidly than predicted by RNA turnover, possibly by shedding of a phosphatidylinositol-linked form[12]. Leu-8 loss on activation is consistent with serological studies showing that Leu-8 is a marker of resting lymphocytes in peripheral lymph nodes[13].

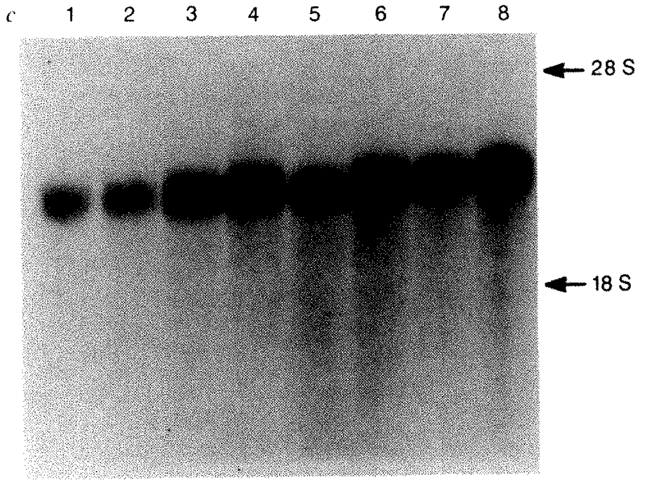
The tissue distributions of Leu-8 (refs 14–20) and Mel-14 antigen[9,23] are similar, with one exception[24,25]. Although Leu-8 is found on almost all medullary and scattered cortical thymocytes[26], histological studies have shown Mel-14 reactivity only on scattered cortical cells[27]. On this basis it has been proposed that murine cortical thymocytes are mature[27]; flow cytometry, however, has shown that most mature thymic cells are Mel-14$^+$ (ref. 28). The present data support the view that the failure to detect Mel-14 reactivity on thymic medullary cells *in situ* is an unusual histological artefact[28].

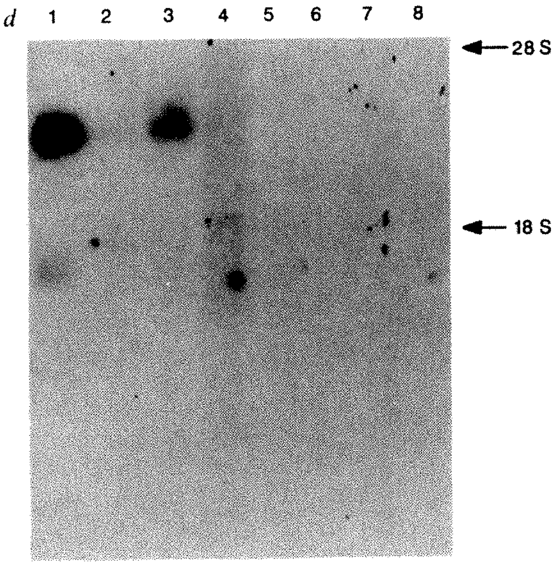
Among human peripheral blood T cells, the CD4$^+$ Leu-8/TQ1$^-$ subset bears almost all of the cells providing help for B cell IgM and IgG synthesis[8,14], whereas CD4$^+$ Leu-8$^+$ cells directly inhibit pokeweed mitogen-induced IgG synthesis[29]. Because T cells that promote immunoglobulin synthesis might be expected to target to, or reside in, peripheral nodes, the finding that CD4$^+$ Leu-8$^-$ cells provide B-cell help is unexpected. One explanation may be that this subset lacks Leu-8 because it consists of activated cells which have exited the nodes into the peripheral circulation. Consistent with this, the mitotically dormant paracortical and interfollicular areas of peripheral nodes bear Leu-8$^+$ T cells, whereas the actively proliferating B-cell germinal centres contain scattered Leu-8$^-$ T cells[26]. Another explanation for why CD4$^+$ Leu-8$^-$ cells provide B-cell help may be that the peripheral pool of cells providing help for immunoglobulin synthesis mainly arises (under normal physiological circumstances) from gut lymphatic structures, where chronic antigenic stimulation ensures a constant supply of activated T cells.

Because both Leu-8$^+$ and Leu-8$^-$ cells can be found among CD4$^+$ cells proliferating in response to a recall antigen, Leu-8 antigen loss following activation may be reversible on at least a subset of cells, and some memory cells may be Leu-8$^+$ (ref. 30). The CD4$^+$ Leu-8$^-$ population contains all of the cytotoxic









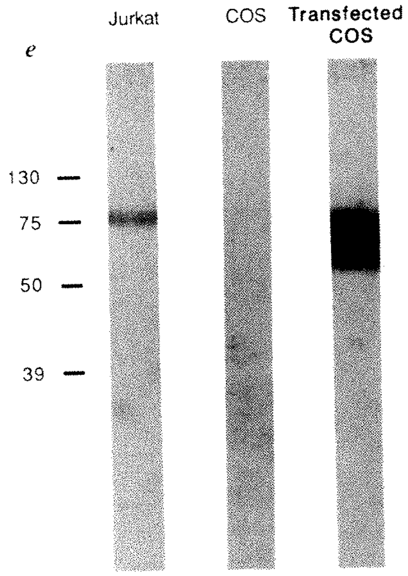


FIG. 3 Leu-8 protein and nucleic-acid analyses. *a*, DNA blot analysis of human DNA digested with *Bam*HI (lane 1), *Eco*RI (lane 2), *Hin*dIII (lane 3) and *Pst*I (lane 4) using a single-stranded Leu-8 probe. *b–e*, RNA blot analysis of total human RNA with a Leu-8 probe. *b*, Effect of activation on Leu-8 RNA expression by peripheral blood lymphocytes: lane 1, resting lymphocytes; lane 2, lymphocytes stimulated with 5 μg ml^{-1} phytohaemagglutinin plus 1 ng ml^{-1} phorbol myristate acetate (PMA); lane 3, lymphocytes stimulated with 5 μg ml^{-1} concanavalin A plus 1 ng ml^{-1} PMA; lane 4, lymphocytes stimulated with pokeweed mitogen (GIBCO, 1:100); lane 5, lymphocytes stimulated with 1 ng ml^{-1} PMA. *c*, RNA from resting and stimulated B cells: lane 1, B cell-enriched peripheral blood lymphocytes stimulated with 1 μg ml^{-1} γ-interferon (Hoechst); lane 2, unstimulated B cell-enriched resting peripheral blood lymphocytes; lane 3, purified tonsillar B cells stimulated with 5 μg ml^{-1} G28-8; lane 4, purified tonsillar B cells stimulated with 5 μg ml^{-1} anti-CD20 antibody 1F5; lane 5, resting purified tonsillar B cells; lane 6, purified tonsillar B cells stimulated with 1 μg ml^{-1} γ-interferon; lane 7, purified tonsillar B cells stimulated with 20 ng ml^{-1} PMA; lane 8, purified tonsillar B cells stimulated with 50 μg ml^{-1} anti-IgM antibody; *d*, T- and myeloid cell line RNAs. Lane 1, HSB-2; lane 2, Molt4; lane 3, Hut78; lane 4, HL60; lane 5, HL60 stimulated with 50 μg ml^{-1} PMA; lane 6, U937; lane 7, U937 stimulated with 50 μg ml^{-1} PMA; lane 8, ThP-1. *e*, Protein blot transfer analysis of Leu-8. Jurkat (T cell leukaemia) cells, COS cells, or COS cells transfected with the longer Leu-8 clone were solubilized in a lysis buffer containing 1% NP-40 and fractionated on 10% SDS polyacrylamide gels. The fractionated proteins were transferred to nitrocellulose electrophoretically. The filter replica was blocked with gelatin (1% in Tris-buffered saline), treated with anti-Leu-8 ascites (1:10,000), washed, and incubated with goat anti-mouse IgG antibody coupled to alkaline phosphatase (Bio-Rad, 1:1000). Bands were visualized by incubation with nitroblue tetrazolium-bromo-chloroindolyl phosphate (Bio-Rad).

METHODS. DNA and RNA blot hybridizations were carried out as previously described[1,2]. Tonsillar B cells were prepared by teasing the tissue in sterile phosphate-buffered saline. Tonsillar or peripheral blood mononuclear cells were washed several times and T cells were removed by incubating with anti-CD3 monoclonal antibody at 4 °C followed by rabbit complement (Pel-Freez) at 37 °C for 45 min in serum-free IMD medium (GIBCO). Monocytes were removed by adherence to plastic dishes, twice for 30 min. 80–85% of the resulting cells were sIgM-positive.

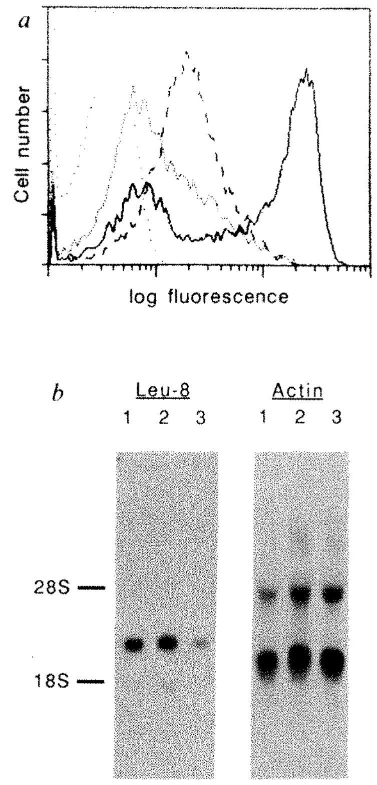


FIG. 4 Leu-8 RNA and surface expression following activation. Peripheral blood lymphocytes were stimulated for the times indicated with 5 μg ml^{-1} phytohaemagglutinin (PHA) and 1 ng ml^{-1} PMA. *a*, Flow cytometric analysis of Leu-8 antigen on cells exposed to 0, 12 and 24 h of stimulation. ——, Untreated; – – –, PHA+PMA (12 h); ·····, PHA+PMA (24 h); · · ·, no stain. *b*, RNA blot analysis of the cells shown in *a*, with a Leu-8 cDNA probe. Lane 1, no treatment; lane 2, stimulated for 12 h; lane 3, stimulated for 24 h. *c*, Hybridization of the blot in *b* with an actin cDNA probe.

(class II restricted) effector cells, whereas only the CD4$^+$ Leu-8$^+$ population proliferates in long-term culture without exogenous cytokine, indicating that this population provides autocrine T-cell help[30]. As the Leu-8 phenotype of these populations is stable on continued culture *in vitro*[30], the CD4$^+$ Leu-8$^-$ cytotoxic/immunoglobulin helper cells may be destined to invade the mucosal lymphatics or extralymphoid sites of inflammation, whereas the CD4$^+$ Leu-8$^+$ autocrine helper cells recirculate through peripheral nodes. ☐

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1. Seed, B. & Aruffo, A. *Proc. natn. Acad. Sci. U.S.A.* **84,** 3365–3369 (1987).
2. Aruffo, A. & Seed, B. *Proc. natn. Acad. Sci. U.S.A.* **84,** 8573–8577 (1987).
3. Lasky, L. A. *et al. Cell* **56,** 1045–1055 (1989).
4. Siegelman, M. H., van de Rijn, M. & Weissman, I. L. *Science* **243,** 1165–1172 (1989).
5. Bowen, B., Nguyen, T. & Lasky, L. *J. Cell Biol.* **109,** 421–427 (1989).
6. Siegelman, M. H. & Weissman, I. L. *Proc. natn. Acad. Sci. U.S.A.* **86,** 5562–5566 (1989).
7. Tedder, T. F. *et al. J. exp. Med.* **170,** 123–133 (1989).
8. Reinherz, E. L. *et al. J. Immun.* **128,** 463–468 (1982).
9. Gallatin, W. M., Weissman, I. L. & Butcher, E. C. *Nature* **304,** 30–34 (1983).
10. Siegelman, M. *et al. Science* **231,** 823–829 (1986).
11. St. John, T. *et al. Science* **231,** 845–850 (1986).
12. Ferguson, M. A. J. & Williams, A. F. *A. Rev. Biochem.* **57,** 285–320 (1988).
13. Poletti, A., Manconi, R. & De Paoli, P. *Hum. Pathol.* **19,** 1001–1007 (1988).
14. Gatenby, P. A., Kansas, G. S., Xian, C. Y., Evans, R. L. & Engleman, E. G. *J. Immun.* **129,** 1997–2000 (1982).
15. Lanier, L. A. *et al. Immun. Rev.* **74,** 143–160 (1983).
16. Lanier, L. L. & Loken, M. R. *J. Immun.* **132,** 151–156 (1984).
17. Kelly, J., O'Farrelly, C., O'Mahoney, C., Weir, D. G. & Feighery, C. *Clin. exp. Immun.* **68,** 177–188 (1987).
18. James, S. P., Fiocchi, C., Graeff, A. S. & Strober, W. *Gastroenterology* **91,** 1483–1489 (1986).
19. Kansas, G. S., Wood, G. S., Fishwild, D. M. & Engleman, E. G. *J. Immun.* **134,** 2995–3002 (1985).
20. Kansas, G. S., Wood, G. S. & Engleman, E. G. *J. Immun.* **134,** 3003–3006 (1985).
21. Lewinsohn, D. M., Bargatze, R. F. & Butcher, E. C. *J. Immun.* **138,** 4313–4321 (1987).
22. Lefrancois, L. *J. Immun.* **138,** 3375–3384 (1987).
23. Schmitz, M., Nunez, D. & Butcher, E. C. *Gastroenterology* **94,** 576–581 (1988).
24. Reichert, R. A., Gallatin, W. M., Weissman, I. L. & Butcher, E. C. *J. exp. Med.* **157,** 813–827 (1983).
25. Kraal, G., Hardy, R. R., Gallatin, W. M., Weissman, I. L. & Butcher, E. C. *Eur. J. Immun.* **16,** 829–834 (1986).
26. Hsu, S.-M. & Jaffe, E. S. *Am. J. Pathol.* **121,** 69–78 (1985).
27. Reichert, R. A., Gallatin, W. M., Butcher, E. C. & Weissman, I. L. *Cell* **38,** 89–99 (1984).
28. Shortman, K., Wilson, K., Van Ewijk, W. & Scollay, R. *J. Immun.* **138,** 342–351 (1987).
29. Kanof, M. E., Strober, W. & James, S. P. *J. Immun.* **139,** 49–54 (1987).
30. Takada, S., Koide, J. & Engleman, E. G. *J. Immun.* **142,** 3038–3044 (1989).

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Interleukin-2-dependent autocrine proliferation in T-cell development

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ACTIVATED T lymphocytes proliferate in response to interleukin-2 (IL-2), which binds to a specific high-affinity receptor (IL-2R)[1–3]. This consists of at least two noncovalently linked polypeptides, p55/IL-2Rα (Tac)[4] and p75/IL-2Rβ[5–7]. Both molecules bind IL-2 independently, with low and intermediate affinity respectively[5–10], but only IL-2Rβ is thought to mediate IL-2 signal transduction[8,11–13]. Although IL-2Rβ seems to be constitutively expressed on resting T lymphocytes[9,14], the growth of these T cells is specifically induced by antigenic triggering by the T-cell receptor (TCR), which then results in the transcription of both IL-2 and IL-2Rα genes[1,2,15]. By contrast, activation of the IL-2/IL-2R pathway in the thymus seems to precede the appearance of the TCR, as IL-2Rα is expressed on T-cell precursors lacking TCR[16–21]. The basis for IL-2R expression by immature thymocytes, however, remains largely unknown. We show here that IL-2Rα-negative T-cell precursors constitutively express IL-2Rβ and produce their own IL-2. The IL-2/IL-2Rβ interaction on these cells induces the expression of IL-2Rα, leading to high-affinity IL-2R display and cellular proliferation. We suggest that this IL-2-dependent autocrine pathway of growth stimulation plays a key role in the intrathymic development of mature T cells.

Adult human thymocytes expressing the CD7 T-cell antigen, but lacking the CD1, CD2, CD3, CD4 and CD8 molecules (pro-T cells), comprise the precursors of IL-2Rα^+ thymocytes and mature T cells[21,22]. Although very few pro-T cells express IL-2Rα (<1% Tac$^+$; Fig. 1*a*), Scatchard analyses of ^{125}I-labelled IL-2 binding indicates that there are ~3,250 IL-2 binding sites per cell (assuming a uniform distribution of sites) (Fig. 2*a*). A large majority (3,000 sites per cell) bound IL-2 with the 'intermediate' affinity characteristic of IL-2Rβ ($K_d \sim 1.9$ nM)[7,8], whereas only 250 high-affinity receptors per cell ($K_d \sim 26$pM) were detected. Parallel studies conducted in the presence of saturating concentrations (500-fold molar excess) of anti-IL-2Rα monoclonal antibody, showed that pro-T cells displayed 2,320 sites per cell ($K_d \sim 850$pM), other than IL-2Rα (Fig. 2*b*). This suggests that the IL-2Rβ intermediate-affinity receptor is the chief IL-2-binding component expressed on pro-T cells. By contrast, an almost threefold lower number of IL-2Rβ was detected in highly purified, resting peripheral T cells (960 sites per cell, $K_d \sim 820$pM) (Fig. 2*c*).

According to these data, freshly isolated pro-T cells contained virtually no detectable IL-2Rα messenger RNA (Fig. 3*a*). Incubation of pro-T cells with doses of recombinant IL-2 (rIL-2) favouring intermediate-affinity binding (1nM), however, resulted in a rapid induction of IL-2Rα gene transcription, which reached maximal levels after eight days (similar to those observed in mitogen-activated peripheral T cells, Fig 3*a*) This induction led to the expression of IL-2Rα molecules on most cultured pro-T cells (86%, Fig 1*b*) A similar proportion of IL-2Rα^+ cells (with a reduced fluorescence intensity, probably secondary to the antibody treatment) was observed when a 300-fold molar excess of anti-IL-2Rα monoclonal antibody was added to the cultures (81%, Fig 1*c*) Binding of IL-2 to individual IL-2Rβ molecules may, therefore, be an effective signal for the induction of IL-2Rα on T-cell precursors The observation that expression in both culture conditions of IL-2Rα was detectable on 5 to 30% of cells after only 24 h of culture, when the cellular recovery was ~70%, formally rules out overgrowth of a minor contaminating subset of IL-2Rα^+ pro-T cells

The functional role of the IL-2/IL-2Rβ interaction on pro-T cells indicated that IL-2 might be available in the thymus at an early developmental stage Indeed, analysis of IL-2 gene expression revealed that the levels of IL-2 mRNA found in freshly isolated pro-T cells were similar to those found in mitogen-activated T lymphocytes, whereas IL-2 transcripts were undetectable in resting peripheral T lymphocytes (Fig 3*a*)

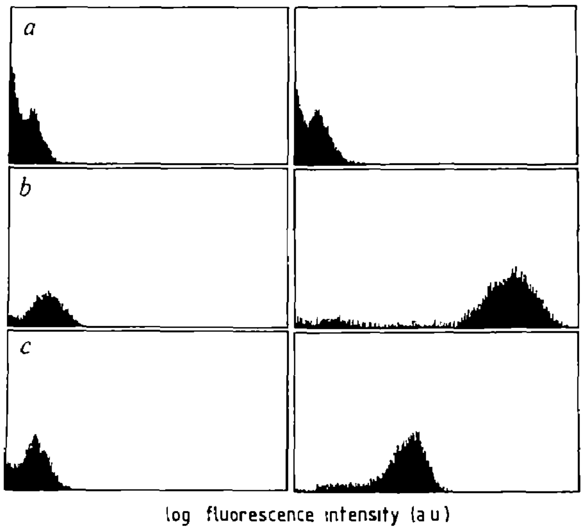

FIG 1 IL-2 induced expression of IL-2Rα in human pro-T cells Pro-T cells, either freshly isolated, *a*, or cultured during 8 days with 1 nM human rIL-2, *b*, or with 1 nM rIL-2 and a 300-fold molar excess of anti-IL-2Rα-monoclonal antibody, *c*, were analysed for the expression of IL-2Rα (right panel) Staining with an irrelevant antibody gives the background fluorescence (left panel)

METHODS Normal human thymocytes were obtained from thymus fragments removed during corrective cardiac surgery of patients of aged one month to three years Intrathymic stem cells, referred to as pro-T cells[22], were isolated by consecutive treatments with anti-CD1, -CD4, -CD8, -CD3 and -CD2 monoclonal antibodies plus rabbit complement (Beringwerke), as previously described[22] Recovered viable cells were treated again, in one step, with a cocktail of the indicated antibodies and incubated for 30 min at 4 °C with magnetic beads coated with affinity-purified sheep anti-mouse IgG (Dynabeads M-450, Dynal) This procedure allowed the isolation of a highly purified preparation of pro-T cells (>99% CD1$^-$2$^-$3$^-$4$^-$8$^-$) Pro-T cell cultures were set up in 24-well macroplates (Costar) (2 × 10^6 cells ml^{-1}) in RPMI-1640 medium (GIBCO) containing 10% FCS (GIBCO) and supplemented with either 1 nM human rIL-2 (Hoffman-La Roche) (cellular recovery ~500%) or with 1 nM rIL-2 plus a 300-fold molar excess of affinity-purified anti-IL-2Rα (H-108)[26] monoclonal antibody (cellular recovery ~300%) Either freshly isolated or cultured pro-T cells were washed twice in RPMI-1640 medium, incubated for 3 min at 4 °C in 0 15 M NaCl, 0 1 M glycine (pH 4) (glycine buffer) and washed again three times to remove bound anti-IL-2Rα antibody molecules Cells were then sequentially incubated with 10 μg ml^{-1} of biotin-conjugated anti-IL-2Rα (H-108) or irrelevant isotype-matching mouse monoclonal antibodies and phycoerythrin-conjugated streptavidin Quantitation of the surface staining of 5 × 10^4 viable cells was performed in an EPICS Profile flow cytometer (Coulter Electronics) The figure shows the results of one representative experiment out of four

Thus, IL-2 may be spontaneously produced by pro-T cells, allowing the acquisition of IL-2Rα '*in vivo*'

To characterize the functional status of the IL-2-induced IL-2Rα molecules, binding assays were performed with pro-T cells, after culture with 1 nM rIL-2 As compared with freshly isolated pro-T cells (Fig 2*a*), cultured pro-T cells showed a 20-fold increase in the expression of low affinity (IL-2Rα) receptors (21,200 sites per cell, $K_d \sim 19$ nM, Fig 2*d*) More importantly, a marked induction of high-affinity binding sites was also detected (980 sites per cell, $K_d \sim 12$ pM, Fig 2*d*) Binding of IL-2 to constitutive IL-2Rβ on pro-T cells leads to the activation of the IL-2Rα gene and results in the assembly of the high-affinity IL-2Rα/β heterodimer These results were confirmed at the protein level by chemical crosslinking performed under high-affinity conditions (with 150 pM ^{125}I-labelled IL-2) Two major IL-2-associated bands of $M_r \sim 90,000$ (90 K) and 70K, corresponding to IL-2Rβ and IL-2Rα, respectively, were detected in cultured pro-T cells (Fig 3*b*), whereas no crosslinked species were detected when pro-T cells were incubated with a 500-fold molar excess of either anti-IL-2Rα monoclonal antibody or unlabelled rIL-2 (Fig 3*b*)

The proliferative potential of pro-T cells was investigated in culture by using a broad concentration range of rIL-2 (1-10^5 pM), with or without saturating concentrations of anti-IL-

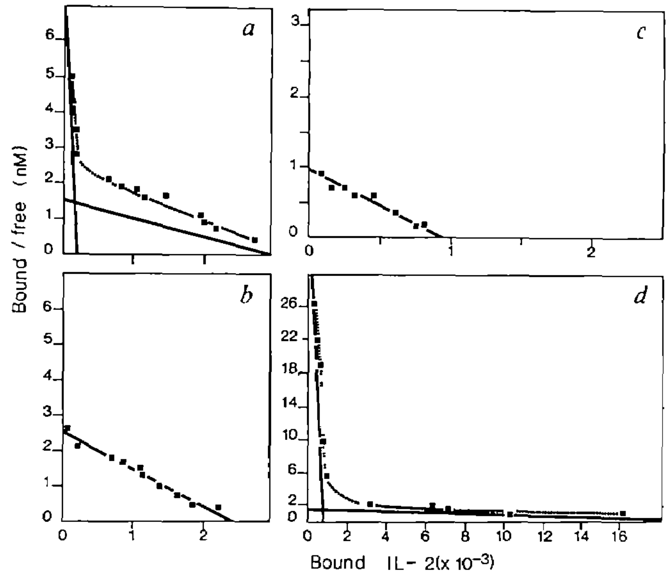

FIG 2 Quantitative analysis of IL-2 binding sites displayed by freshly isolated pro-T cells, resting peripheral T cells and IL-2 cultured pro-T cells IL-2 binding assays were performed in freshly isolated pro-T cells (*a*, *b*), in pro-T cells cultured with 1 nM rIL-2 for 8 days (*d*) and in resting peripheral T cells (*c*), either in the absence (*a*, *d*) or in the presence (*b*, *c*) of anti-IL-2Rα

METHODS Pro-T cells were obtained as described in Fig 1 legend, and highly purified resting peripheral T cells (96% CD2$^+$, <0 1% Tac$^+$, <0 5% CD16$^+$) were isolated from peripheral blood by nylon wool columns followed by treatment with anti-CD16 and anti-IL-2Rα antibodies and incubation with magnetics beads, as described in Fig 1 legend Aliquots of 2 × 10^7 cells were extensively washed and treated with glycine buffer as in Fig 1 to remove bound IL-2 molecules Cells (10^6) were then incubated with serial dilutions of ^{125}I-labelled human rIL-2 (1 pM–25 nM final concentration) (Amersham) for 1 h at 4 °C, in the absence or presence of a 500-fold molar excess of affinity-purified anti-IL-2Rα (H-108) antibody, as previously described[2,3] Specifically bound and free ^{125}I-labelled IL-2 were determined by solid scintillation counting The calculated values for the dissociation constant (K_d) and the number of binding sites per cell were derived by Scatchard computer analysis[27] of equilibrium binding data (solid lines) The dashed lines represent a computer-generated best fit to the experimental data The data were corrected for nonspecific binding of IL-2, determined in the presence of a 250-fold molar excess of unlabelled rIL-2, and for the loss of ligand binding activity incurred during the IL-2 labelling procedures The data are representative of three independent experiments

2Rα antibodies (3×10^{-7} M, Fig 4) In the absence of anti-IL-2Rα, IL-2 induced the proliferation of pro-T cells in a dose-dependent manner, maximal proliferation being achieved at doses of rIL-2 favouring intermediate-affinity binding (10 nM, Fig 4*a*) Also, blocking of IL-2Rα molecules did not affect cell proliferation at doses of rIL-2 high enough to saturate IL-2Rβ (<1 nM), whereas a significant inhibition was observed at low doses of rIL-2 (<500 pM), sufficient to bind to high-affinity IL-2Rα/β heterodimers (Fig 4*a*) By contrast, a fifteenfold lower concentration of rIL-2 was required to induce maximal proliferation of activated T cells expressing high-affinity IL-2R (100% Tac$^+$) and, therefore, a shift in the IL-2 dose-response curve was induced by anti-IL-2Rα Ten- to fifteenfold higher concentrations of IL-2, sufficient to saturate the IL-2Rβ, were then required to induce T-cell growth (Fig 4*c*) Similar inhibition patterns were observed for resting T lymphocytes (Fig 4*b*), supporting the reported IL-2-dependent induction of high-affinity receptors on these cells[14] The discrete proliferation observed in resting T cells when compared with pro-T cells (Fig 4) may reflect their lower expression of IL-2Rβ (900 compared with 2,300 sites per cell)

Taken together, these results indicate that both intermediate- and high-affinity IL-2R can be involved in the IL-2-promoted proliferation of pro-T cells, indicating that, before acquisition of IL-2Rα/β, IL-2Rβ alone can mediate signal transduction of IL-2 The observation that even without exogenous IL-2 supplementation, pro-T cells developed proliferative responses which can be blocked by anti-IL-2Rα monoclonal antibody, however, argues that the spontaneous and 'physiological' growth of these cells may be mediated mainly by the high-affinity form of the IL-2R Our data, therefore, provide evidence for the involvement of an IL-2-dependent autocrine pathway in the intrathymic development of mature T cells As previously proposed[23], such a nonspecific activation of polyclonal growth would operate selectively early in T-cell development, given the constitutive expression of mature IL-2 mRNA by pro-T cells In agreement with this, high levels of IL-2 transcripts have been detected in 15-day fetal thymocytes, whereas expression decreases dramatically by day 16 up until birth[24]

Although the activation signals inducing IL-2 gene expression within the thymus are as yet unknown, recent results indicate that cellular interactions involving thymic stromal components and production of lymphokines other than IL-2, such as IL-1, play an essential role in this process[25] Thus, IL-1 appears to induce a marked activation of IL-2 gene in precursor thymocytes (E Rothenberg, personal communication) In addition, we have observed that both IL-1 and IL-3 can regulate IL-2Rα expression on pro-T cells (unpublished data) Although the possibility that this induction results from endogenous IL-2 production in the culture cannot be ruled out, it seems likely that more than one cytokine pathway may allow the acquisition of an IL-2-responsive state which, in turn, would ensure polyclonal expansion of T-cell precursors and T-cell differentiation The autocrine behaviour that we propose occurs in T-cell differentiation could possibly be a common developmental strategy for other haemopoietic lineages □

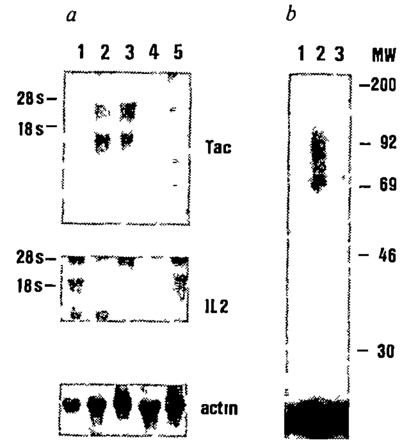

FIG 3 Constitutive expression of IL-2 transcripts in T-cell precursors and induction of IL-2Rα gene expression Acquisition of high-affinity IL-2Rα/β induced by IL-2/IL-2Rβ interactions *a*, Northern blot analysis of RNA obtained from freshly isolated pro-T cells (lane 1), phytohaemagglutinin-activated normal peripheral T cells (lane 2), pro-T cells cultured with 1 nM rIL-2 for 8 days (lane 3), JY B cells (lane 4) and resting peripheral T cells (lane 5) RNA (12 μg) was loaded on a formaldehyde-agarose gel, transferred to nylon membranes and hybridized with the ^{32}P-labelled (nick-translated) Tac-2A probe[28], the IL-2 probe[29], or an actin probe used as control, as previously described[22] *b*, Chemical crosslinking of ^{125}I-labelled IL-2 to IL-2-treated pro-T cells Pro-T cells, cultured for 8 days with 1 nM rIL-2 (10^7 cells) and washed with glycine buffer as described in Fig 2 legend, were incubated with ^{125}I-labelled human rIL-2 (150 pM) in the absence (lane 2) or presence of a 500-fold molar excess of either unlabelled rIL-2 (lane 1) or anti-IL-2Rα (H-108) monoclonal antibodies (lane 3) Disuccinimidyl suberate (Pierce) (0 3 nM) was used to covalently crosslink ^{125}I-labelled IL-2 to the surface of intact cells as previously described[9] Cells were solubilized in 1% SDS, 5% 2-mercaptoethanol sample buffer and analysed on 8% SDS-polyacrylamide gels Similar results were obtained in four independent experiments

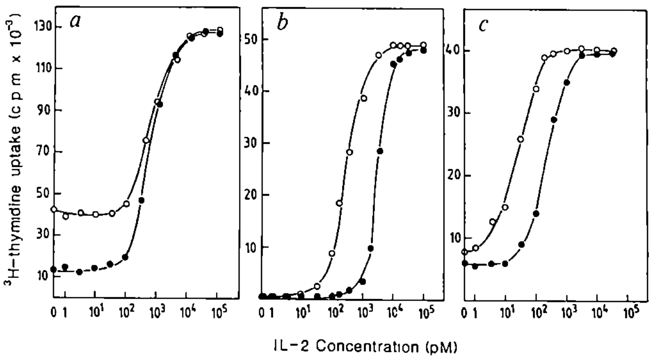

FIG 4 IL-2 induced proliferation of pro-T cells *a*, Pro-T cells, *b*, highly purified resting peripheral T cells, and *c*, phytohaemagglutinin-activated T cells were cultured in flat-bottomed 96-well microtitre plates (Costar) in RPM1-1640 medium at 10^6 cells ml^{-1} (*a*, *b*) or 10^5 cells ml^{-1} (*c*) with $3\,0\times10^{-7}$ M normal mouse affinity-purified IgG (○) or anti-IL-2Rα (H-108) antibody (●), in the presence of different concentrations of rIL-2 (1–10^5 pM) Proliferative responses were analysed by [^{3}H]thymidine incorporation at day 5 of culture, after addition of 1 μCi per well of [^{3}H]thymidine (Amersham) for the last 18 h Each value represents the mean of triplicate cultures Standard deviations were ≤10%

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1 Smith K A *Immunol Rev* **51**, 335-357 (1980)
2 Robb R J Munck A & Smith K A *J exp Med* **154**, 1455-1474 (1981)
3 Robb R J Greene W C & Rusk C M *J exp Med* **160**, 1126-1146 (1984)
4 Leonard W J *et al Nature* **300**, 267-269 (1982)
5 Sharon M Klausner R D Cullen B R Chizzonite R & Leonard W J *Science* **234**, 859-863 (1986)
6 Tsudo M Kozak R W Goldman C K & Waldmann T A *Proc natn Acad Sci USA* **83**, 9694-9698 (1986)
7 Teshigawara K Wang H Kato K & Smith K A *J exp Med* **165**, 223-238 (1986)
8 Wang H M & Smith K A *J exp Med* **166**, 1055-1069 (1987)
9 Dukovich M *et al Nature* **327**, 518-522 (1987)
10 Robb R J Rusk C M Yodoi J & Greene W C *Proc natn Acad Sci USA* **84**, 2002-2006 (1987)
11 Robb R J & Greene W C *J exp Med* **165**, 1201-1206 (1987)
12 Smith K A *Immun Today* **9**, 36-37 (1988)
13 Tanaka T Saiki O Doi S Negoro S & Kishimoto S *J Immun* **140**, 470-473 (1988)
14 Bich Thuy L T *et al J Immun* **139**, 1550-1556 (1987)
15 Meuer S C *et al Proc natn Acad Sci USA* **81**, 1509-1513 (1984)
16 Raulet D H *Nature* **314**, 101-103 (1985)
17 Ceredig R Lowenthal J W Nabholz M & MacDonald H R *Nature* **314**, 98-100 (1985)
18 de la Hera A *et al Eur J Immun* **16**, 653-658 (1986)
19 Shimonkevitz R P Husmann L A Bevan M J & Crispe I N *Nature* **329**, 157-159 (1987)
20 Jenkinson E J Kingston R & Owen J J T *Nature* **329**, 160-162 (1987)
21 Toribio M L *et al Immunol Rev* **104**, 55-79 (1988)
22 Toribio M L *et al J exp Med* **168**, 2231-2249 (1988)

23 de la Hera A Toribio M L Marcos M A R Marquez C & Martinez-A C *Eur J Immun* **17**, 683-687 (1987)
24 Carding S R *et al Proc natn Acad Sci USA* **86**, 3342-3345 (1989)
25 de la Hera A Toribio M L & Martinez-A C *International Immun* (in the press)
26 Carrera A C Sanchez-Madrid F Lopez-Botet M Bernabeu C & O de Landazuri M *Eur J Immun* **17**, 179-186 (1987)
27 Munson P J & Rodbard D *Analyt Biochem* **107**, 220-239 (1980)
28 Nikaido T *et al Nature* **331**, 631-635 (1984)
29 Taniguchi T *et al Nature* **302**, 305-310 (1983)

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A glycophospholipid anchor is required for Qa-2-mediated T cell activation

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A NUMBER of lymphocyte surface proteins are anchored in the cell membrane by glycophosphatidyl inositol (known as GPI) linkages instead of hydrophobic protein domains[1,2] Treatment of mouse T lymphocytes with antibodies specific for two such proteins, Thy-1 and Ly-6, are known to induce proliferation[3,4] We have found that antibodies specific for Qa-2, a GPI-anchored class I histocompatibility antigen[5], can also activate mouse T cells. To determine whether the GPI-anchor is important for this pathway of cell activation, we produced transgenic mice expressing either normal GPI-anchored Qa-2, or Qa-2 molecules with a membrane-spanning protein domain derived from H-2 Our studies show that only lymphocytes from transgenic mice carrying GPI-anchored forms of Qa-2 can be activated *in vitro* by Qa-2-specific antibodies. We also show that transgenic mouse T cells expressing a GPI-anchored form of H-2D^b can be activated by anti-H-2D^b antibodies These results strongly indicate that the GPI-anchor is critical for this pathway of T cell activation

Activation of T cells is normally the result of binding of antigen receptors to antigenic peptides presented in association with major histocompatibility antigens A number of other T-cell membrane proteins (for example, CD2, CD3 and CD28) can also induce activation in response to specific antibodies[6-8] These antibodies may therefore mimic the binding of signalling molecules to their natural ligands Molecules such as CD2 are transmembrane proteins that require a cytoplasmic domain to function in signalling[9] Some GPI-anchored membrane proteins, such as Thy-1, have also been shown to act as signalling molecules, despite the fact that the fatty acid anchoring group appears to associate with only one face of the lipid bilayer[2,10] This indicates the existence of a second type of transmembrane signal delivery pathway involving GPI-anchors[11]

In mice, the class I, H-2 K, D and L major histocompatibility antigens are transmembrane glycoproteins that are thought to present processed endogenous antigenic fragments to receptors on T cells Antibodies against H-2 are not normally mitogenic for T cells but antibodies specific for Qa-2, a GPI-anchored class I molecule[5], are Purified T cells from C57BL/10, Qa-2$^+$, mice were stimulated to divide by three Qa-2 specific monoclonal antibodies T cells from BALB/cJ mice, which express fewer Qa-2 molecules, could also be activated under these conditions Anti-immunoglobulin antibody and phorbol myristate acetate (PMA), an activator of protein kinase C, were both required for cell division to occur Specificity for Qa-2 could be demonstrated by examining the proliferative responses of T cells from recombinant mouse strains B6 K1 and B6 K2, which differ only in the *Q* region[12] of the major histocompatibility complex T cells from B6 K2 mice, which express Qa-2, could be activated by Qa-2 specific antibodies, but T cells from B6 K1 mice, which lack the antigen, could not (Table 1) Both types of T cells, however, respond equally well to Thy-1 specific antibodies

To determine whether the GPI membrane anchor is required for the triggering of T cells by Qa-2 specific antibodies, we constructed hybrid class I genes by recombining segments of $Q9^b$ or $Q7^b$, which both encode GPI-anchored Qa-2 molecules[13-15], with corresponding regions of $H\text{-}2D^b$ The products of hybrid genes *QD1* and *QD3* are shown in Fig 1 A GPI-anchored Qa-2 molecule is encoded by *QD1*, and *QD3* encodes a variant form of Qa-2 which spans the cell membrane using a transmembrane segment derived from H-2D^b Transgenic mice were derived by introducing these constructs into fertilized oocytes from CBA/Ca mice, which lack the genes for Qa-2 As shown in Fig 2, spleen cells from both CBA/Ca QD1 and CBA/Ca QD3 transgenic mice expressed molecules reactive with anti-Qa-2 antibodies QD1 molecules could be removed from cell membranes by treatment with phosphatidylinositol-specific phospholipase C (PtdIns-PLC), indicating the presence of a GPI anchor, but QD3 molecules were completely resistant to the enzyme

To confirm that QD3 molecules have a transmembrane orientation, we treated microsomal membranes from biosynthetically labelled CBA/Ca QD3 spleen cells with proteinase K Cytoplasmic domains of transmembrane proteins exposed on the microsomal surface immediately after synthesis are removed by the enzyme[16] Gel electrophoresis shows that QD3 molecules were reduced in relative molecular mass by ~3,000 following proteinase K treatment, consistent with removal of the H-2D^b-derived cytoplasmic domain[16] By contrast, the size of QD1 was unchanged (data not shown) This confirms that QD3 spans the membrane Both QD1 and QD3 were found to be associated with beta-2 microglobulin, as are Qa-2 and H-2D^b (data not shown)

The proliferative responses of T cells from CBA/Ca, CBA/Ca QD1 and CBA/Ca QD3 mice treated with Thy-1 or

TABLE 1 Proliferation of mouse T cells in response to Qa-2 specific antibodies

Cells	Antibody	Specificity	[^{3}H]thymidine incorporation (c p m)
C57BL/10	None	—	600
	Qa-m2	Qa-2	48,000
	Qa-m7	Qa-2	67,000
	D3 262	Qa-2	14,000
	H142 45	H-2K^b, D^b	<600
	B22 249	H-2D^b	<600
	K9 136	H-2K^b	<600
BALB/c	None	—	3,400
	Qa-m2	Qa-2	56,000
	KT16	Thy-1	104,000
B6 K1	Qa-m2	Qa-2	300
	KT16	Thy-1	66,000
B6 K2	Qa-m2	Qa-2	23,900
	KT16	Thy-1	68,000

T lymphocytes were isolated from mouse spleens by nylon wool fractionation followed by absorption to anti-immunoglobulin coated dishes Cells were then incubated with stimulatory antibodies at 1 μg ml^{-1} for 30 min at 25 °C, washed then cultured for 48 h in 96-well costar plates at 5×10^5 cells per well in the presence of rabbit anti-mouse immunoglobulin F(ab')$_2$ fragments (5 μg ml^{-1}) and PMA (50 ng ml^{-1}) Cell cultures were pulsed with [^{3}H]thymidine (1 μCi, for 2 h), collected and counted

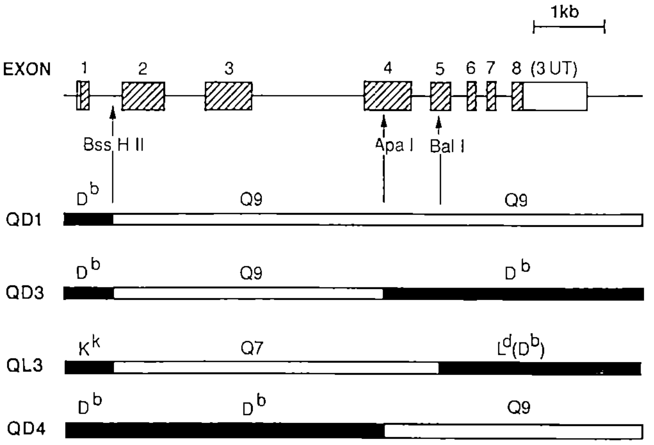

FIG 1 Construction of *H-2/Qa-2* hybrid genes Subclones of *H-2D^b*, *Q7^b* and *Q9^b* genes were recombined at the *Bss*HII, *Apa*I and *Bal*I sites indicated to construct the hybrid genes Plasmid sequences were removed before injection into pronuclei of fertilized CBA/Ca mouse embryos using standard procedures Transgenic *QD1*, *QD3*, and *QL3* mice were identified by hybridization of DNA from tail biopsy to a probe for the *Q9* large intron (between exons 3 and 4) *QD4* mice were identified using a 0 5 kb *Bam*HI fragment of *H-2D^b* from the 5′ flanking region[20] UT, untranslated

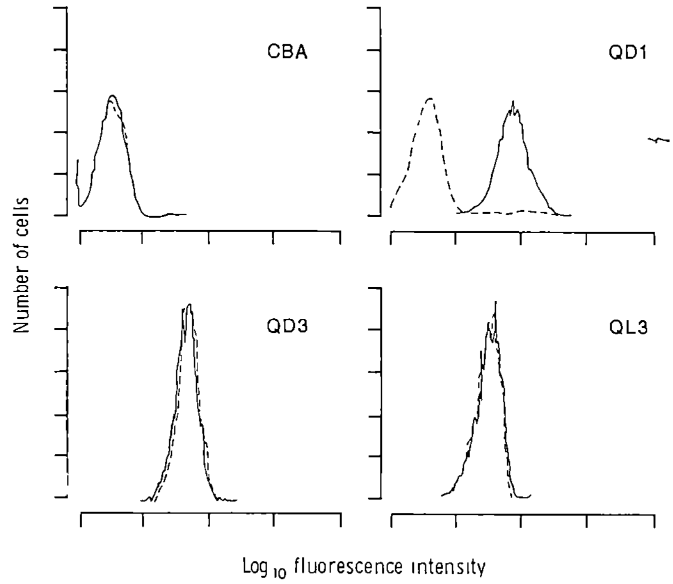

FIG 2 Flow cytometry of hybrid class I molecules on transgenic T cells T cells from transgenic mouse spleens were gated using biotinylated L3T4 and Ly-2 specific antibodies which were developed with streptavidin phycoerythrin Transgene products on T cells were stained using Qa-m2 antibody and rabbit antimouse immunoglobulin conjugated to fluorescein isothocyanate Solid lines show untreated cells, broken lines show cells after treatment with PtdIns-PLC before addition of antibody Conjugate-only controls were indistinguishable from CBA/Ca profiles and therefore are not shown

Qa-2 specific antibodies are shown in Table 2 Antibodies specific for Qa-2 were mitogenic for T cells from CBA/Ca QD1, but not CBA/Ca or CBA/Ca QD3 mice Proliferation of CBA/Ca QD1 cells was abrogated by pretreatment with PtdIns-PLC T cells from all three mouse lines responded well to Thy-1 specific antibodies, confirming that this activation pathway is not distorted by the presence of the transgene or its product These results suggest that binding of anti-Qa-2 antibodies is only mitogenic for T cells if the target antigen is anchored in the membrane with a GPI linkage

In addition to their membrane anchors, QD1 and QD3 differ by eight amino acids in their membrane-proximal alpha-3 protein domains To investigate whether T cells expressing membrane-spanning class I molecules with extracellular domains entirely of Qa-2 origin would proliferate in response to anti-Qa-2 antibodies, we produced transgenic mice expressing another construct, *QL3* As shown in Fig 1, the entire extracellular section of QL3 is derived from Qa-2 The remainder of the molecule, including part of the membrane-spanning segment and the cytoplasmic domain, is from H-2L^d QL3 molecules were stably expressed as transmembrane glycoproteins on the surface of transgenic T cells (Fig 2), despite the presence of an intramembrane aspartic acid residue, encoded by *Q7^b*, which was previously shown to be important for the addition of the GPI anchor[17] T cells from CBA/Ca QL3 and CBA/Ca QD3 transgenic mice behaved similarly in the proliferation assay Both gave a strong proliferative response to anti Thy-1 but no response to anti-Qa-2 antibodies As the entire extracellular portions of QD1 and QL3 differ by only a single amino acid at position 173 (alpha-2 domain) owing to a single base change between *Q7* and *Q9* (ref 15), this strongly suggests that it is the GPI anchor that endows QD1 with the ability to deliver activation signals

If GPI membrane anchors can confer transmembrane signalling capability on membrane proteins it should be possible to create new signal delivery structures by attaching phospholipid anchors to normally non-mitogenic molecules To test this we produced one further transgenic mouse line, CBA/Ca QD4, which expresses a GPI-anchored derivative of the H-2D^b

TABLE 2 Response of transgenic mouse T cells to class I-specific antibodies

Experiment 1 Antibody	Specificity	[^{3}H]Thymidine incorporation (c p m) CBA/Ca	QD1	QD3
–	–	260 (260)	340 (540)	300 (270)
KT16	Thy-1	35,400 (6,400)	73,000 (34,200)	95,400 (11,700)
Qa-m2	Qa-2	170 (70)	28,670 (147)	80 (90)
Experiment 2		C57BL/10	QL3	QD4
–	–	ND	2,400	6,800
KT16	Thy-1	124,600 (40,800)	116,000 (152,000)	70,100 (34,000)
Qa-m2	Qa-2	29,800 (7,300)	2,200 (2,100)	5,100 (ND)
141 30	H-2D^b	5,100 (6,800)	5,700 (ND)	25,400 (10,300)

Conditions as for Table 1 In parentheses, cells were pretreated with 0 1U ml^{-1} *Bacillus thuringiensis* PtdIns-PLC (1 h, 37 °C) and washed before adding stimulatory antibody molecule (Fig 1) We found that T cells from these animals could be activated by antibodies against H-2D^b (Table 2) The antibody used, H141 30, is specific for the two outermost protein domains of H-2D^b (ref 18) This was demonststrated by a lack of reactivity with cells carrying H-2L^d (ref 18), which has an identical sequence to H-2D^b in the alpha-3 domain Antibody H141 30 does not cross-react with Qa-2, as indicated by its lack of reactivity with CBA/Ca QD1 spleen cells (data not shown) and did not cause proliferation of C57BL/10 T cells, which express conventional H-2D^b molecules (Table 2)

These activation data can best be explained by involvement of phospholipid membrane anchors in the process of transmembrane signalling and thus constitute direct evidence of a biological function associated with a GPI membrane anchor Other potential attributes of GPI linkages, such as increased lateral mobility of proteins in the plane of the membrane and their potential for selective release by phospholipases[1,2], have not yet been shown to have functional importance By contrast, there is evidence suggesting the involvement of GPI anchors in cell activation[11] First, treatment of lymphocytes with PtdIns-PLC reduces their response to mitogens[5] Second, T cell lines defective in anchor biosynthesis show reduced lymphokine production when stimulated with antigen[19] The precise mechanism by which GPI anchors participate in cell triggering phenomena is still unclear, but their involvement in the activation of T lymphocytes suggests that they may be important for the regulation of cell proliferation during the immune response ☐

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1 Low M *Biochem J* **244,** 1-13 (1987)
2 Ferguson M A J & Williams A F *A Rev Biochem* **57,** 285-320 (1988)
3 Kroczek R A Gunther K C Seligmann B & Shevach E M *J Immun* **136,** 4379-4384 (1986)
4 Yeh E T H Reiser H Daley J & Rock K L *J Immun* **138,** 91-97 (1987)
5 Stiernberg J Low M G Flaherty L & Kincade P W *J Immun* **138,** 3877-3884 (1987)
6 Ledbetter J D *et al Eur J Immun* **18,** 1601-1608 (1988)
7 Van Wauwe J P De May J R & Goossens J G *J Immun* **124,** 2708-2713 (1980)
8 Hara T Fu S M & Hansen J A *J exp Med* **161,** 1513-1524 (1985)
9 He Q Beyers A D Barclay A N & Williams A F *Cell* **54,** 979-984 (1988)
10 Gunther K C *et al Nature* **326,** 505-507 (1987)
11 Robinson P J & Spencer S C *Immun Lett* **19,** 85-94 (1988)
12 Flaherty L *Immunogenetics* **3,** 533-539 (1976)
13 Waneck G L Shermar D H Calvin S Allen H & Flavell R A *J exp Med* **165,** 1358-1370 (1987)
14 Mellor A L Antoniou J & Robinson P J *Proc natn Acad Sci USA* **82,** 5920-5924 (1985)
15 Devlin J J Weiss E H Paulson M & Flavell R A *EMBO J* **4,** 3203-3207 (1985)
16 Dobberstein B Garoff H Warren G & Robinson P *Cell* **17,** 759-769 (1979)
17 Waneck G L Stein M E & Flavell R A *Science* **241,** 697-699 (1988)
18 Lemke H Hammerling G J & Hammerling U *Immunol Rev* **47,** 175-206 (1979)
19 Yeh E T H Reiser H, Bamezai A & Rock, K L *Cell* **62,** 665-674 (1988)
20 Weiss E H *et al Nature* **310,** 650-655 (1984)

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Purified inositol 1,4,5-trisphosphate receptor mediates calcium flux in reconstituted lipid vesicles

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INOSITOL 1,4,5-trisphosphate (Ins (1,4,5)P_3), a second messenger molecule involved in actions of neurotransmitters, hormones and growth factors, releases calcium from vesicular non-mitochondrial intracellular stores[1] An Ins(1,4,5)P_3 binding protein, purified from brain membranes[2], has been shown to be phosphorylated by cyclic-AMP-dependent protein kinase[3] and localized by immunohistochemical techniques to intracellular particles associated with the endoplasmic reticulum[4] Although the specificity of the Ins(1,4,5)P_3 binding protein for inositol phosphates and the high affinity of the protein for Ins(1,4,5)P_3 indicate that it is a physiological Ins(1,4,5)P_3 receptor mediating calcium release, direct evidence for this has been difficult to obtain Also, it is unclear whether a single protein mediates both the recognition of Ins(1,4,5)P_3 and calcium transport or whether these two functions involve two or more distinct proteins. In the present study we report reconstitution of the purified Ins(1,4,5)P_3 binding protein into lipid vesicles We show that Ins(1,4,5)P_3 and other inositol phosphates stimulate calcium flux in the reconstituted vesicles with potencies and specificities that match the calcium releasing actions of Ins(1,4,5)P_3 These results indicate that the purified Ins(1,4,5)P_3 binding protein is a physiological receptor responsible for calcium release

The Ins(1,4,5)P_3 receptor protein was solubilized using CHAPS as a detergent, rather than Triton X-100 which has been used previously[2], because Triton detergent is not compatible with the dialysis technique of vesicle reconstitution[5,6] The Ins(1,4,5)P_3 receptor protein was purified as previously described[2] with minor modifications (see Fig 1) In the reconstitution experiments purified Ins(1,4,5)P_3 receptor protein was added to a mixture of CHAPS, solubilized phosphatidylcholine and phosphatidylserine, and the mixed micelles of protein and lipid were dialysed for 48 hr against a 1,000-fold excess of buffer To determine whether the Ins(1,4,5)P_3 binding protein was incorporated into vesicles, we fractionated the vesicles on a continuous sucrose gradient and monitored [^{3}H]Ins(1,4,5)P_3 binding as well as levels of [^{14}C]phosphatidylcholine, which we used as a marker for the lipid vesicles (Fig 1*b*) The [^{3}H] Ins(1,4,5)P_3 binding activity and [^{14}C]phosphatidylcholine co-migrated on the gradient and nearly 100% of the binding activity was recovered Total protein concentration also co-migrated with the peak of [^{3}H]Ins(1,4,5)P_3 binding activity (data not shown) Pure Ins(1,4,5)P_3 binding protein, dialysed in the absence of lipid, appeared to aggregate as it migrated to the densest portion of the gradient (data not shown) Lipid vesicles without receptor protein appeared slightly less dense than the protein containing vesicles (data not shown) Analysis of protein within the vesicles by SDS-PAGE revealed a single band with a relative molecular mass of 260,000 (260K) (Fig 1*a*), the same as purified Ins(1,4,5)P_3 receptor protein not incorporated into vesicles[2] Thus, the reconstitution procedure results in the incorporation of Ins(1,4,5)P_3 binding protein into the vesicles with no change in molecular weight, and the protein retains its ability to bind Ins(1,4,5)P_3

The properties of [^{3}H]Ins(1,4,5)P_3 binding in the reconstituted vesicles were essentially the same as those of the purified unreconstituted protein[2] Unlabelled Ins(1,4,5)P_3 inhibited [^{3}H]Ins(1,4,5)P_3 binding 50% at ~40nM (Fig 2) Of a variety of inositol phosphates examined, Ins(1,4,5)P_3 was the most potent inhibitor of binding with Ins(2,4,5)P_3 being the next potent Both Ins(1,3,4)P_3 and Ins(1,3,4,5)P_4 were much less potent inhibitors of binding, whereas other inositol phosphates examined were essentially inactive (Table 1) Heparin, a known inhibitor of Ins(1,4,5)P_3 binding[7] and Ins(1,4,5)P_3-mediated

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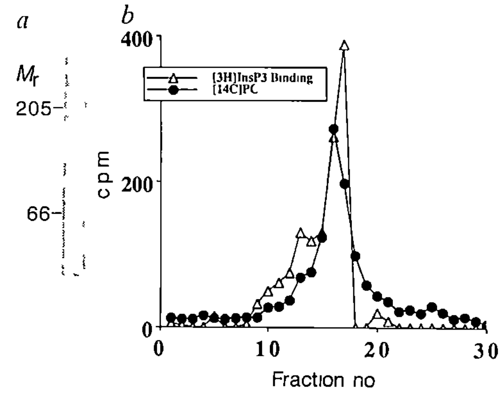

FIG 1 Reconstitution of purified Ins(1,4,5)P_3 receptor protein *a*, SDS-PAGE analysis of protein in reconstituted lipid vesicles (7 5% gel, Coomassie Brilliant Blue stain) *b*, Disposition of specific [^{3}H]Ins(1,4,5)P_3 (open triangles) binding in continuous sucrose gradient (5–20%) fractionation of reconstituted proteoliposomes Fraction number 1 corresponds to 20% sucrose or the bottom of the gradient, whereas fraction number 30 is 5% sucrose at the top of the gradient The [^{14}C]Phosphatidylcholine (PC, closed circles) was used as a tracer for the lipid vesicles Nonspecific [^{3}H]Ins(1,4,5)P_3 binding was measured in the presence of 1 μM Ins (1,4,5)P_3 This experiment was performed three times with the same results Binding was determined in duplicate or triplicate with replicates varying <10%

METHODS Ins(1,4,5)P_3 receptor was purified as described[2] with the following modifications CHAPS (1%, Sigma) was used as the detergent to allow dialysis for reconstitution, and the receptor was purified using only two affinity column chromatography steps[2] Partially purified receptor was obtained by fractionating CHAPS solubilized proteins on Heparin agarose (Sigma, Type II) as described[2] The purified receptor was obtained by fractionating the proteins which elute from heparin agarose on concanavalin sepharose[2] The reconstitution strategy was based on a previously described procedure used for the nicotinic acetylcholine receptor[6] Solubilized liposomes were prepared by sonicating (for 12–20 min in a cylindrical bath sonicator) a mixture of phosphatidylcholine (in the presence or absence of tracer amounts of [^{14}C] phosphatidylcholine) and phosphatidylserine (Avanti Polar Lipids) at a ratio of 3 1, and a lipid concentration of 40 mg ml^{-1} and in a buffer (buffer A) containing 50 mM NaCl, 50 mM KCl and 20 mM Tris-HCl (pH 7 4, 25 °C) Following sonication the lipid concentration was reduced to 20 mg ml^{-1} by adding an equal volume of buffer A and CHAPS was added to bring the final detergent concentrations to 1% This lipid mixture was then mixed with purified Ins(1 4,5)P_3 receptor (100 μg ml^{-1} protein) or partially purified (400 μg ml^{-1}) at a ratio of 1 1 After incubation on ice for 20 min, the mixture was dialysed (molecular mass cutoff, 10,000) against a 1,000-fold excess of buffer A supplemented with 1 mM EDTA, 1 mM EGTA and 2 mM 2-mercaptoethanol The buffer was changed every 8 h for 48 h to effect detergent removal and vesicle formation These vesicles were then loaded onto a 5–20% continuous sucrose gradient (30 ml) and centrifuged overnight (SW 28, 20,000 r p m (140,000*g*) Beckman Ultracentrifuge) Fractions (1 ml) were collected, [^{14}C]phosphatidylcholine content and [^{3}H]Ins(1,4,5)P_3 binding were measured Protein concentration was determined by the method of Bradford[22], and SDS-PAGE was by the method of Laemmli[23]

calcium release[8–13], completely inhibited binding at 100 μg ml^{-1} (Table 1) As previously reported for the purified, unreconstituted Ins(1,4,5)P_3 binding protein[2], calcium (1 mM) had no effect on [^{3}H]Ins(1,4,5)P_3 binding—which was markedly influenced by pH, more than doubling as pH was increased from 7 4 to 8 8 (data not shown)

To monitor calcium flux, reconstituted vesicles were incubated with $^{45}Ca^{2+}$ in the presence or absence of various concentrations of Ins(1,4,5)P_3 or other inositol phosphates (Figs 2,3, Table 1) At 10 μM Ins(1,4,5)P_3 the rate of $^{45}Ca^{2+}$ flux was stimulated at least fivefold, being half maximal within 4 s and complete within 10 s (Fig 3) The very rapid kinetics of Ins(1,4,5)P_3-stimulated $^{45}Ca^{2+}$ influx would be expected for ion channels reconstituted in small unilamellar liposomes[6] Half maximal and maximal Ins(1,4,5)P_3-stimulated $^{45}Ca^{2+}$ influx required ~40 nM and 1 μM Ins(1,4,5)P_3, respectively, very similar to corresponding potencies for inhibiting [^{3}H]Ins(1,4,5)P_3 binding (Fig 2) The relative effects of other inositol phosphates on $^{45}Ca^{2+}$ flux also resembled their influences on [^{3}H]Ins(1,4,5)P_3 binding, with Ins(1,4,5)P_3 being most potent and Ins(2,4,5)P_3 the only other inositol phosphate with activity (Table 1) These effects resemble the relative calcium-releasing potencies of inositol phosphates in several systems[14–16] Also, the relative potencies of the inositol phosphates in mediating $^{45}Ca^{2+}$ release from cerebellar microsomes were the same as in the reconstituted vesicles (data not shown) Interestingly, whereas Ins(1,3,4)P_3 and Ins(1,3,4,5)P_4, at concentrations of 10 and 20 μM respectively, produced significant inhibition of [^{3}H]Ins(1,4,5)P_3 binding, they did not elicit a calcium flux (Table 1) Whether these results indicate that at those concentrations these substances are Ins(1,4,5)P_3 receptor antagonists is not clear Heparin (100 μg ml^{-1}) completely blocked the ability of Ins(1,4,5)P_3 (1 μM) to stimulate a $^{45}Ca^{2+}$ flux (Table 1) with no effect on $^{45}Ca^{2+}$ flux in the absence of Ins (1,4,5)P_3

Although in these experiments we examined the effects of inositol phosphates on the influx of $^{45}Ca^{2+}$ into reconstituted vesicles, in other experiments we have shown similar effects on $^{45}Ca^{2+}$ release from reconstituted vesicles preloaded with $^{45}Ca^{2+}$ Ins(1,4,5)P_3 doses of 10 μM or 1 μM released, with rapid kinetics, the $^{45}Ca^{2+}$ from the inside of vesicles preloaded by freeze thaw (see Fig 3 legend) When the partially purified preparations of the receptor (see Fig 1 legend) were reconstituted in parallel with the purified receptor, the specific activity of [^{3}H]Ins(1,4,5)P_3 binding increased 9 3-fold after the second affinity chromatography step, whereas the specific activity of Ins(1,4,5)P_3-mediated calcium flux increased 10 3-fold In

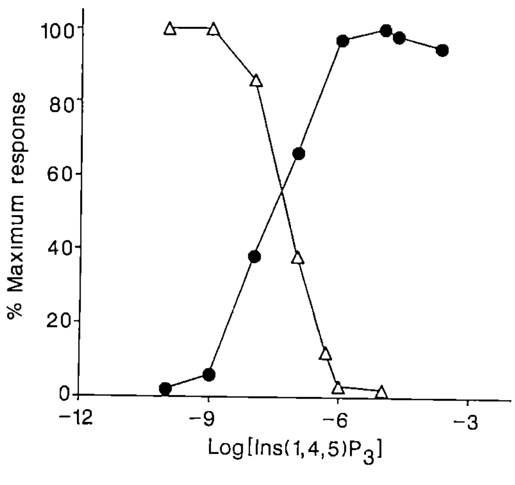

FIG 2 Concentration-response relationships for Ins(1,4,5)P_3 inhibition of [^{3}H]Ins(1,4,5)P_3 binding (△) and stimulation of $^{45}Ca^{2+}$ influx into vesicles (●) reconstituted from purified Ins(1,4,5)P_3 receptor protein Calcium flux was measured as described in the legend to Fig 3, and the incubation time for calcium flux was 10 s This experiment has been performed four times with essentially the same results Measurements were made in triplicate or quadruplicate with replicates varying <10%

experiments using lipid vesicles without incorporated Ins(1,4,5)P_3 receptor protein, no effects of inositol phosphates on the $^{45}Ca^{2+}$ flux were detected Also, reconstitution of protein from column fractions containing no binding activity resulted in vesicles that had no Ins(1,4,5)P_3-stimulated $^{45}Ca^{2+}$ transport To demonstrate that the intact vesicle structure is required for Ins(1,4,5)P_3 effects on calcium flux, we solubilized reconstituted vesicles with CHAPS (1%) and were no longer able to detect Ins(1,4,5)P_3-stimulated $^{45}Ca^{2+}$ flux In addition we examined whether the $^{45}Ca^{2+}$ accumulated in the reconstituted vesicles after Ins(1,4,5)P_3 stimulation could be released by ionophores We therefore collected the vesicles after passing them over the Dowez column, incubated them in the presence and absence of the calcium ionophore A23187 and then passed them over a second Dowex column All of the $^{45}Ca^{2+}$ accumulated after Ins(1,4,5)P_3 stimulation was released by the ionophore

Our ability to reconstitute Ins(1,4,5)P_3-stimulated calcium flux into lipid vesicles containing purified Ins(1,4,5)P_3 binding protein establishes that this protein is a physiological receptor mediating Ins(1,4,5)P_3 effects on calcium release The close correlation between potency of inositol phosphates to influence ligand binding and calcium flux supports this conclusion Our findings also establish that a single protein mediates both recognition of Ins(1,4,5)P_3 and the stimulation of calcium flux In this way the Ins(1,4,5)P_3 receptor resembles neurotransmitter-gated ion channels, such as the nicotinic acetylcholine[6], GABA (γ-aminobutyric acid)-benzodiazepine[17], and glycine[17] receptors, in which a single protein-complex contains recognition sites both for the neurotransmitter and associated ion channels These neurotransmitter receptors are comprised of several distinct subunits, whereas the Ins(1,4,5)P_3 receptor seems to be a tetramer of four identical subunits[2] The neurotransmitter receptors are integral proteins of the plasma membrane with recognition sites on the extracellular surface, whereas the Ins(1,4,5)P_3 receptor is a novel intracellular receptor with the recognition site for Ins(1,4,5)P_3 on the cytoplasmic side In this way the Ins(1,4,5)P_3 receptor resembles the recently characterized[18-20] and cloned[21] ryanodine receptor, which mediates calcium flux from the sarcoplasmic reticulum of skeletal muscle The Ins(1,4,5)P_3 receptor also resembles the ryanodine receptor in having a high relative molecular mass, and a similar proposed subunit structure and chromatographic behaviour[18-21] It is likely, therefore, that these two receptors are members of a new class of intracellular ligand-gated ion channels ☐

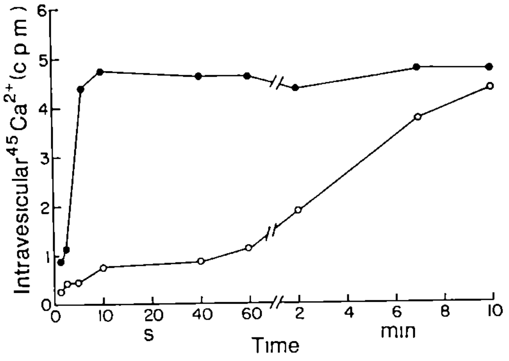

FIG 3 Time course of $^{45}Ca^{2+}$ flux in vesicles reconstituted with purified Ins(1,4,5)P_3 receptor Open symbols, accumulation of $^{45}Ca^{2+}$ in reconstituted vesicles in the absence of any agonist, closed symbols, $^{45}Ca^{2+}$ accumulated in the presence of 10 μM Ins(1,4,5)P_3 Data are expressed as c p m (×1,000) This experiment has been replicated three times with similar results Measurements were made in duplicate or triplicate with replicates varying <10%

METHODS Following reconstitution of the purified Ins(1,4,5)P_3 receptor as described in the legend to Fig 1, vesicles were dialysed overnight in buffer A supplemented with 2 mM 2-mercaptoethanol to remove the calcium chelators which interfere with calcium flux measurements and to bring the final calcium concentration to nominally free levels of ~10 μM free calcium as measured by a calcium sensitive macroelectrode Calcium flux was determined using tracer amounts of $^{45}Ca^{2+}$ and cation exchange chromatography Calcium influx was measured by incubating 85 μl of reconstituted vesicles with 2 μCi $^{45}Ca^{2+}$ in the presence or absence of 10 μM Ins(1,4,5)P_3 The final reaction volume was 100 μl After incubation for the indicated times, the flux reaction was stopped with a threefold excess of buffer A supplemented with 0 3 mM $CaCl_2$, 5 mM $MgSO_4$, 1 mM $CdCl_2$ and 100 μg ml^{-1} heparin (Sigma) (Stop buffer) This 400 μl mixture of vesicles and stop buffer was immediately loaded onto a 3 ml column of Tris-Dowez (Sigma, Dowez 50-W X-8, pH 8 0) which had been equilibrated with buffer containing 200 mM sucrose, 20 mM Tris (pH 7 4, 25 °C) and 0 3% BSA The column was immediately washed with 3 5 ml of the same buffer (without BSA) to wash the vesicles and the intravesicular $^{45}Ca^{2+}$ through the column The $^{45}Ca^{2+}$ content of the vesicles was determined by liquid scintillation spectrometry In some experiments, however, following the removal of extravesicular calcium by the Dowez column, the vesicles were immediately incubated in 3 5 ml of the sucrose containing buffer in the presence or absence of 10 μM A23187, a calcium selective ionophore After a 10 min incubation at 22 °C, the vesicles were reloaded onto (and washed through) a second Dowex column (6 ml column volume) to determine the selective releasibility of the accumulated calcium For efflux measurements vesicles were loaded with $^{45}Ca^{2+}$ by rapid freeze thaw After freeze thaw, vesicles were diluted fivefold into buffer A in the presence or absence of 10 μM Ins(1,4,5)P_3 After a 10 s incubation at 22 °C the flux reaction was stopped and the intravesicular $^{45}Ca^{2+}$ content was determined as described above

TABLE 1 [^{3}H]Ins(1,4,5)P_3 binding and Ins(1,4,5)P_3-stimulated $^{45}Ca^{2+}$ flux

Compound	Concentration (μM)	% Inhibition of [^{3}H]Ins(1,4,5)P_3 binding	% Stimulation of $^{45}Ca^{2+}$ flux
Ins(1,4,5)P_3	1	100	97
Ins(1,3,4)P_3	10	40	0
Ins(2,4,5)P_3	2	72	52
Ins(2)P	5	3	0
Ins(4)P	5	0	0
Ins(1,4)P_2	10	5	0
Ins(1,3,4,5)P_4	20	62	0
InsP_5	10	12	0
InsP_6	10	15	0
Heparin	100 μg ml^{-1}	100	0
Ins(1,4,5)P_3 +heparin	1 100 μg ml^{-1}	—	3

Calcium flux and [^{3}H]Ins(1,4,5)P_3 binding were determined as described in the legends to Figs 1 and 2 Flux measurements were made after a 10 s incubation as in Fig 3 These experiments have been performed three times in triplicate or quadruplicate with replicates varying by <15%

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1 Berridge M J *A Rev Biochem* **56**, 159-193 (1987)
2 Supattapone S Worley P F Baraban J M & Snyder S H *J biol Chem* **263**, 1530-1534 (1988)
3 Supattapone S *et al Proc natn Acad Sci USA* **85**, 8747-8750 (1988)
4 Ross C A *et al Nature* **339**, 468-470 (1989)
5 Jones O T Earnest J P & McNamee M G in *Biological Membranes A Practical Approach* (eds Findlay J B C & Evans W H (IPL Oxford 1987)
6 Huganir R & Racker E *J biol Chem* **257**, 9372-9378 (1982)
7 Worley P F *et al J biol Chem* **262**, 12132-12136 (1987)
8 Hill T Berggren P O & Boynton A *Biochem biophys Res Commun* **149**, 897-901 (1987)
9 Cullen P J Comerford J G & Dawson A P *FEBS Lett* **228**, 57-59 (1988)
10 Nilsson T Zwiller J Boynton A L & Berggren P O *FEBS Lett* **229**, 211-214 (1988)
11 Joseph S K & Rice H L *Molec Pharm* **35**, 355-359 (1989)
12 Ghosh T K *et al J biol Chem* **263**, 11075-11079 (1988)
13 Guillemette G Lamontagne S Boulay G & Mouillac B *Molec Pharm* **35**, 339-344 (1989)
14 Ghosh T K Mullaney M M Tarazi F I & Gill D L *Nature* **340**, 236-239 (1989)
15 Irvine R F *Biochem Soc Trans* **17**, 6-9 (1989)
16 Nahorski S R *Trends pharm Sci* **10**, 139-144 (1989)
17 Barnard E A Darlison M G & Seeburg P *Trends Neurosci* **10**, 502-509 (1987)
18 Inui M Saito A & Fleischer S *J biol Chem* **262**, 1740-1747 (1987)
19 Lai G A Erickson H P Roussear D Liu Q-Y & Meissner G *Nature* **331**, 315-319 (1988)
20 Imagana T Smith J S Coronado R & Campbell K P *J biol Chem* **262**, 16636-16643 (1987)
21 Takeshima H *et al Nature* **339**, 439-445 (1989)
22 Bradford M M *Analyt Biochem* **72**, 248-254 (1976)
23 Laemmli U K *Nature* **227**, 680-685 (1970)

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Nature and site of phospholamban regulation of the Ca^{2+} pump of sarcoplasmic reticulum

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THE rapid removal of Ca^{2+} ions from the cytosol, necessary for the efficient relaxation of cardiac muscle cells, is performed by the Ca^{2+}-pumping ATPase of the sarcoplasmic reticulum The calcium pump is activated by cyclic AMP- and calmodulin-dependent phosphorylation of phospholamban, an integral membrane protein of the sarcoplasmic reticulum. Using a heterobifunctional crosslinking agent which can be cleaved and photoactivated, we provide evidence for a direct interaction between the two proteins Only the non-phosphorylated form of phospholamban interacts with the ATPase, demonstrating that phospholamban is an endogenous inhibitor that is removed from the ATPase by phosphorylation Non-phosphorylated phospholamban interacts only with the calcium-free conformation of the ATPase and is released when it is converted to the calcium-bound state We localized the site of interaction to a single peptide isolated after cyanogen bromide cleavage of the ATPase The peptide derives from a domain just C-terminal to the aspartyl phosphate of the active site. This domain is unique to ATPases of the sarcoplasmic reticulum in that it has no homology with any other phosphorylation-type ion pump The domain occurs in both slow- and fast-twitch isoforms of the ATPase, even though phospholamban is not expressed in fast-twitch muscles

Phospholamban (PLN) is a complex of five identical amphipathic peptides of relative molecular mass (M_r) ~6,000 (ref 1) In its hydrophilic domain each monomer contains adjacent serine and threonine residues that can be selectively phosphorylated by the cAMP-dependent and calmodulin-dependent kinases respectively[2] Phosphorylation of PLN is important in the mechanical response of cardiac muscle to stimulation by beta-agonists[3,4] We isolated phospholamban oligomer[5] and conjugated the ε-amino group of its single lysine residue, situated near the N-terminus in the hydrophilic domain, to the *N*-oxysuccinimide moiety of the heterobifunctional Denny-Jaffe crosslinking reagent[6] to form a stable amide linkage When conjugated with the Denny-Jaffe reagent, PLN remains oligomeric The reagent contains a cleavable distal portion carrying a photoactivatable aryl azide and a radioactive ^{125}I label It has been postulated that PLN, which is present in roughly stoichiometric amounts with the calcium pump (that is, one monomer per pump unit), interacts directly with the enzyme to induce a conformational change in it Light activation of conjugated PLN incubated with purified cardiac sarcoplasmic reticulum (SR) ATPase resulted in the formation of a complex (Fig 1, lane 3) But when PLN was pre-phosphorylated by the cAMP-dependent kinase, or when Ca^{2+} ions were added to the incubation mixture, there was no crosslinking with the ATPase (Fig 1, lanes 1, 2 and 4) Complex formation therefore occurs only when PLN is in the unphosphorylated state Experiments using reconstitution techniques[7], monoclonal antibodies against PLN[8] or controlled tryptic digestion of PLN[9] have indicated that PLN is an inhibitor of the calcium pump and that phosphorylation removes this inhibition, a suggestion that is now strongly supported by our result

Addition of 100 μM Ca^{2+} completely inhibits complex formation between the two proteins (Fig 1) When Ca^{2+} ions bind to high-affinity sites[10], the ATPase undergoes a large conformational change to assume the E_1 conformation, which can then be phosphorylated by ATP during the calcium transport cycle[11] We investigated whether inhibition of the PLN interaction by calcium resulted from conversion of the ATPase into the E_1 state by studying the calcium dependence of the hydrolytic activity of the pump in parallel with labelling experiments The hydrolytic activity can be taken as an indicator of the saturation level of the high-affinity Ca^{2+}-binding sites[11] Figure 2 shows that the activation of the pump by Ca^{2+} is inversely correlated with its ability to interact with PLN, so only the E_2 (Ca^{2+}-free) conformation of the ATPase can bind PLN Interaction with non-phosphorylated PLN therefore shifts the equilibrium of the pump in favour of the E_2 form, slowing down the $E_2 \rightarrow E_1$ conversion and raising the apparent K_m for calcium of the enzyme This would explain earlier observations on the binding kinetics of Ca^{2+} to the cardiac ATPase PLN phosphorylation, which results in the removal of PLN from its binding site on the pump, was shown to increase the rate of binding of Ca^{2+} ions[12] and the apparent affinity for calcium of the ATPase[13,14] Changes in the electrostatic properties of the SR membrane during PLN phosphorylation also contribute to the increased calcium affinity of the ATPase[15]

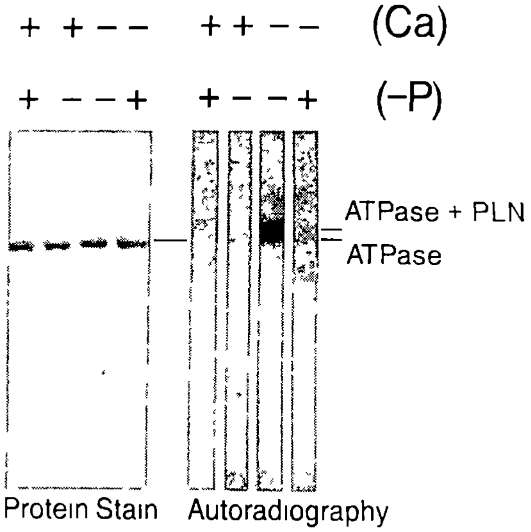
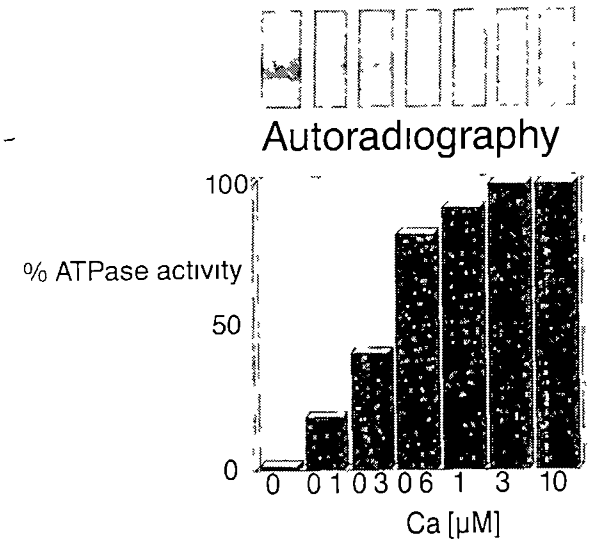


FIG 1 Crosslinking of Denny-Jaffe labelled PLN to the isolated SR ATPase *a*, Protein-stained SDS-polyacrylamide gel *b*, Autoradiograph of same gel Conjugated PLN crosslinked to the SR Ca^{2+}-ATPase (lane 3) The interaction between the two proteins was strongly inhibited by pre-phosphorylation of PLN (lanes 1 and 4) or by Ca^{2+} ions (lanes 1 and 2)

METHODS PLN was purified[1] and labelled with the Denny-Jaffe reagent in 0 1% $C_{12}E_8$ detergent (dodecyl octaoxyethylene glycol monoether), 10 mM phosphate buffer, pH 6 8, in the dark[17] Labelling was carried out in the presence of cyclohexadione to block all arginine residues As the N-terminus is blocked, the only group left to react with the reagent is the single lysine of PLN Using cold Denny-Jaffe reagent, about 5 mol azide per PLN pentamer could be incorporated SR ATPase was isolated from rabbit heart according to ref 18 The ATPase preparation (~1 mg ml^{-1}) was crosslinked[17] to the conjugated PLN (stoichiometric ratio 1 1, pump to pentamer) in a medium composed of 20 mM Tris-maleate, pH 7 0, 0 1% $C_{12}E_8$, 2 5 mM $MgCl_2$, and in the presence of either 0 5 mM EGTA (lanes 3 and 4) or 0 1 mM Ca^{2+} (lanes 1 and 2) If necessary, PLN was pre-phosphorylated (lanes 1 and 4) using the catalytic subunit of the cAMP-dependent protein kinase Results were identical when phosphorylation was carried out before or after conjugation of PLN to the Denny-Jaffe reagent Samples of protein (10 μg) were then run on 10% polyacrylamide/SDS-gels[19], stained with Coomassie brilliant blue (*a*), dried and autoradiographed (*b*) The molecular weight of the crosslinked ATPase population was altered by ~6,000 because boiling in sample buffer dissociates the PLN pentamer The sample buffer contained dithiothreitol, which on heating partially reduces the azo crosslink, thus explaining the width of the radioactive band The coupling efficiency was low (5-10%), which is usual in most photo-crosslinking reactions

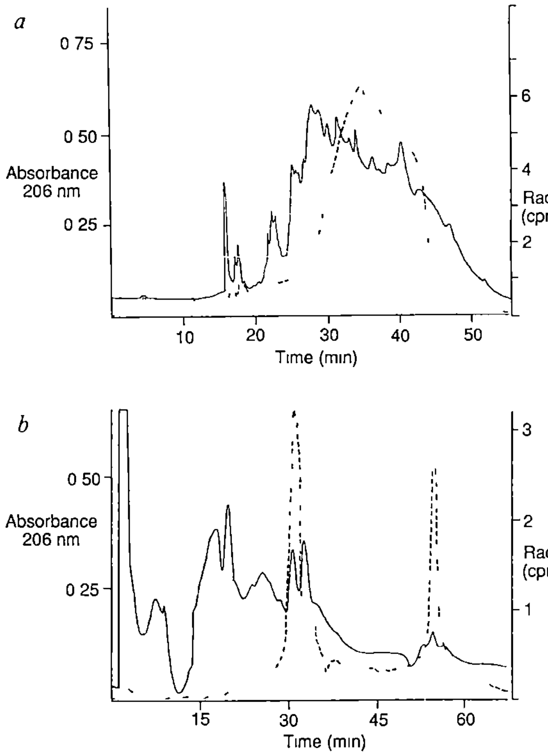


◀ FIG 2 Calcium dependence of PLN binding to the SR ATPase *a*, Autoradiograph of SDS-polyacrylamide gel *b*, Calcium-dependence of ATPase activity Ca^{2+} ions in the submicromolar range strongly inhibit the interaction between PLN and the Ca^{2+}-ATPase (see the Ca^{2+}-dependent decrease in ^{125}I-labelled ATPase–PLN complex in *a*) There is a similar calcium dependence of ATPase activation under identical conditions (*b*) This indicates that the conformational change induced by calcium binding to the set of high-affinity binding sites on the ATPase is responsible for the inability of PLN to bind to the enzyme

METHODS The SR ATPase was crosslinked to non-phosphorylated, Denny-Jaffe-conjugated PLN as described in the legend to Fig 1 Medium was supplemented with 0 5 mM EGTA and different amounts of $CaCl_2$ to give the calculated free Ca^{2+} concentration Calcium dependence of ATPase activity was measured under the same conditions, but in the absence of modified PLN, using a coupled enzyme assay[20]

FIG 3 Isolation and amino-acid sequence analysis of the PLN-binding peptide from the SR ATPase *a*, Reverse-phase HPLC of peptides formed by cyanogen bromide digestion of ^{125}I-labelled SR ATPase *b*, Re-chromatography on an ion-exchange column of the fractions from *a* that contained the radioactivity Solid lines indicate absorbance at 206 nm and broken lines, radioactivity in c p m ▶

METHODS Isolated cardiac SR Ca^{2+}-ATPase (10 mg) was photolysed in the presence of 200 µg non-phosphorylated, Denny-Jaffe-conjugated PLN, as described in the legend to Fig 1 After reduction and carboxymethylation of thiol groups, PLN was cleaved off using 100 mM sodium dithionite[17] and removed from the solution by dialysis (cut-off range 50,000 M_r) This cleavage leaves the ^{125}I label attached to the ATPase domain that interacts with PLN The ATPase was then digested with CNBr and fractionated on a 300-Å, 7-µ, 250 × 10 mm C-18 RP column (0 1% trifluoroacetic acid in water to 100% *n*-propanol in 50 min) The broad radioactive peak was collected and diluted into 20% *n*-propanol, 10 mM Tris-HCl, pH 8 5 (buffer A), and then injected onto a Mono-Q column equilibrated in the same buffer A gradient of 0–0 5 M NaCl in buffer A was run over 60 min Radioactive fractions were desalted by reverse-phase HPLC and sequenced in an Applied Biosystems 470A sequencer with on-line detection Half of the phenylthiohydantoin amino acids released per cycle were injected onto the 120A HPLC unit of the system for analysis, and the other half counted to determine radioactivity in a Beckman gamma counter



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PLB-binding
peptides    slow twitch (rabbit)        FILDKVDGETCSLNEFTITGSTYAPIGEVHKDDKPVKCHQ
            fast twitch (dog)           FIIDKVDGNLCVLNEFAITGSTYAPEGEVLKNDKPIRS

Ca (SR)    1 LGCTSVICSDKTGTLTTNQ  MSVCRMFILDKVDGETCSLNEFTITGSTYAPIGEVHKDDKPVKCHQTDGLVELATICALCNDSALDYNE  AKGVYEKVGEATE
           2 LGCTSVICSDKTGTLTTNQ  MSVCKMFIIDKVDGDFCSLNEFSITGSTYAPEGEVLKNDKPIRSGQFDGLVELATICALCNDSSLDFNE  TKGVYEKVGEATE

Ca (PM)    3 MGNATAICSDKTGTLTMNR  MTVVQAYINEK        HY     KKVPEPEAI   PPNILSYL VTGISVNCA YTSKILPPEK    EGGLPRHVGNKTE

Na/K       4 LGSTSTICSDKTGTLTQNR  MTVAHMWFDNQIHEADTTENQ                SGVSFDKTSATWLALSRIAGLCNRAVFQANQDNLPILKRAVAGDASE
           5 LGSTSTICSDKTGTLTQNR  MTVAHMWFDNQIHEADTTENQ                SGISFDKTSLSWNALSRIAALCNRAVFQAGQDSVPILKISVAGDASE

H          6 MSGVNMLCSDKTGTLTLNK  MEIQEQCFTFEEG                                   NDLKSTLVLAALAAKWREPPRDALDTMVLGAADLDE
           7 LAGVEILCSDKTGTLTKNK  LSLHEIYTVEGVS                                  PDDLMLTAPLAASRKKKGLDAIDKAFLKSLKQYPKAK

K          8 AGDVDVLLLDKTGTITLGN  RQASEFIPAQGVD                                   EKTLADAAQLASLADETPEGRSIVILAKQRFNLRER
           9 ANDLDVIMLDKTGTLTQGK  FTVTGIEILD                                      EAYQEEEILKYIGALEAHANHPLAIGIM  NYLKEK

             PHOSPHORYLATION DOMAIN
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FIG 4 The amino-acid sequence of the ^{125}I-labelled peptides obtained from rabbit cardiac muscle SR ATPase and from dog fast-twitch muscle ATPase is shown aligned with homologous regions in other ATPases (1) Rabbit slow-twitch muscle SR Ca^{2+}-ATPase[21], (2) rabbit fast-twitch muscle SR Ca^{2+}-ATPase[22], (3) human plasma membrane Ca^{2+}-ATPase[23], (4) sheep plasma membrane $Na^+ + K^+$ ATPase [24], (5) torpedo plasma membrane $Na^+ + K^+$ ATPase[25], (6) *Saccharomycas cerevisiae* H^+-ATPase[26], (7) *Neurospora crassa* H^+-ATPase[27], (8) *Escherica coli* K^+-ATPase[28], and (9) *Streptococcus faecalis* K^+-ATPase[29] Despite the high degree of homology found in all ATPases around the phosphorylation domain, the PLN-binding region is unique to the two isoforms of the SR Ca^{2+} pump and is not even found in the plasma membrane (PM) Ca^{2+}-ATPase

The domain of interaction of the ATPase with PLN has been identified Crosslinked ^{125}I-labelled PLN-ATPase complex was cleaved at the azo linkage of the Denny-Jaffe reagent using dithionite This removed PLN from its binding site while the ^{125}I label remained attached to the ATPase After removal of PLN by extensive dialysis, cyanogen bromide digestion of the ATPase gave two peptides, both carrying the ^{125}I label, which must therefore have contained the binding domain The peptides were purified (Fig 3) and sequenced Their molecular weights were identical, as judged from plasma desorption mass spectrometry, and their sequence was the same They differed only in the position of labelling of the two neighbouring lysines (arrows in Fig 4) Figure 4 shows that the peptide(s) originated from a region of the ATPase just C-terminal to the aspartyl residue that is phosphorylated during the transport cycle Binding of unphosphorylated PLN to this site could therefore be interfering with the formation of the high-energy phosphorylated intermediate E_1-P, with its transition to the E_2–P form, and/or with the hydrolysis of E_2–P, which is a rate-limiting step[16]

The Ca^{2+}-ATPase of the SR shares a high degree of homology with all other ATPases of the phosphorylation type In particular, the phosphorylation site is virtually identical in all these pumps (Fig 4) Strikingly, however, the site of PLN interaction is a unique feature of the SR ATPase(s) Thus, regulation by PLN of ATPase activity, other than in the SR type, would not be possible The PLN-binding domain is also found in the fast-twitch muscle isoform of the SR Ca^{2+}-ATPase (Fig 4), so it has been possible also to crosslink PLN to ATPase preparations isolated from dog and rabbit fast skeletal muscle SR The same lysine residues were labelled as in the slow-twitch/cardiac enzyme Independent experiments using PLN affinity column chromatography showed that ATPase from rabbit fast skeletal muscle SR bound to the column only when the ATPase was in the E_2 form (data not shown) Evidently the regulation of the Ca^{2+}-pumping activity in fast- and slow-twitch muscle SR depends on the differential expression of the regulatory protein PLN, rather than on intrinsic structural differences in the ATPase isoforms present in the two muscle types □

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1 Fujii J *et al J clin Invest* **79,** 301-304 (1987)
2 Wegener A D Simmerman H K Liepniesks J & Jones L R *J biol Chem* **261,** 5154-5159 (1986)
3 Lindemann J P Jones L R Hathaway D R Henry B G & Watanabe A M *J biol Chem* **258,** 464-471 (1983)
4 Garvey J L Kranias E G & Solaro R J *Biochem J* **249,** 709-714 (1988)
5 Inui M Kadoma M & Tada M *J biol Chem* **260,** 3706-3715 (1985)
6 Denny J B & Blobel G *Proc natn Acad Sci USA* **81,** 5286-5290 (1984)
7 Inui M Chamberlain B K Saito A & Fleischer S *J biol Chem* **261,** 1794-1800 (1986)
8 Suzuki T & Wang J H *J biol Chem* **261,** 7018-7023 '1986)
9 Kirchberger M A Borchman D & Kasinathan C *Biochemistry* **25,** 5484-5492 (1986)
10 Dupont Y *Biochim biophys Acta* **688,** 75-87 (1982)
11 Inesi G *A Rev Physiol* **47,** 573-601 (1985)
12 Tada M *et al J biol Chem* **255,** 1985-1992 (1980)
13 Tada M Kirchberger M A & Katz A M *J biol Chem* **250,** 2640-2647 (1975)
14 Tada M & Katz A M *A Rev Physiol* **44,** 401-423 (1982)
15 Chiesi M & Schwaller R *FEBS Lett* **244,** 241-244 (1989)
16 Tada M Ohmori F Yamada M & Abe H *J biol Chem* **254,** 319-326 (1979)
17 James P *et al J biol Chem* **263,** 2905-2910 (1988)
18 Van Winkle W B Pitts B J R & Entman M L *J biol Chem* **253,** 8671-8673 (1978)
19 Laemmli U K *Nature* **227,** 680-685 (1970)
20 Neet K E & Green N M *Arch Biophys Biochim* **178,** 588-597 (1977)
21 MacLennan D H Brandl C J Korkzak B & Green N M *Nature* **316,** 696-700 (1985)
22 Brandl C J Green N M Korkzak B & MacLennan D H *Cell* **44,** 595-607 (1986)
23 Verma A K *et al J biol Chem* **263,** 14152-14159 (1988)
24 Shull G E Schwarz A & Lingrel J B *Nature* **316,** 691-695 (1985)
25 Kawakami K *et al Nature* **316,** 733-736 (1985)
26 Serrano R Kielland Brandt M C & Fink G R *Nature* **319,** 689-693 (1986)
27 Addison R *J biol Chem* **261,** 14896-14901 (1986)
28 Hesse J *et al Proc natn Acad Sci USA* **81,** 4746-4750 (1984)
29 Solioz M Matthews S & Furst P *J biol Chem* **262,** 7358-7362 (1987)

Yeast replication factor-A functions in the unwinding of the SV40 origin of DNA replication

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CELL-free replication systems for simian virus 40 (SV40) DNA are taken to be a model for the replication of eukaryotic chromosomes, because only one viral protein is required to supplement the replication proteins provided by a human cell extract. To prove that these cellular proteins function in chromosomal DNA replication we have begun to identify homologous proteins in an organism that can be genetically manipulated Here we report the identification of yeast replication factor-A (yRF-A) from *Saccharomyces cerevisiae* and show that it is functionally and structurally related to a human protein that is required for the initiation and elongation of SV40 DNA replication Yeast RF-A, a multi-subunit phosphoprotein, is similar to the human protein in its chromatographic behaviour, subunit structure and DNA-binding activity The yeast protein will fully substitute for the human protein in an early stage of the initiation of SV40 DNA replication. Substitution of yRF-A in the complete SV40 replication system, however, results in reduced DNA replication, presumably due to a requirement for species-specific interactions between yeast RF-A and the DNA polymerase complex

Human cell extracts that are supplemented with purified SV40 large tumour antigen (T-Ag) support replication of plasmid DNAs containing SV40 origin of replication sequences[1-3] After biochemical fractionation, the crude extract has been replaced by five purified proteins and one partially purified fraction (refs 4–7, see ref 8 for a review) One of these proteins, replication factor-A (RF-A), a multi-subunit protein with single-strand DNA (ssDNA)-binding activity[9-11], is required for the initiation of DNA replication by participating in the formation of an 'unwound complex' on the SV40 DNA template[5 12-14] This complex is an early intermediate in the initiation reaction and accumulates in the absence of dNTP precursors It is detected by the accumulation of a topologically underwound plasmid DNA (form U) after phenol extraction and agarose gel electrophoresis Form U DNA can be obtained in the absence of DNA synthesis by incubation of plasmid DNA containing the SV40 origin of replication with three purified components T-Ag, topoisomerase I and RF-A After the removal of the proteins, the unwound DNA is subjected to electrophoresis in an agarose gel in which form U DNA migrates slightly slower than form I DNA This 'unwinding' assay was used to screen yeast extracts for components with an activity able to replace that of human RF-A This assay was expected to detect heterologous ssDNA-binding proteins because the *Escherichia coli* ssDNA-binding protein (SSB), which functions during the initiation and elongation of *E coli* DNA replication, substitutes for human RF-A in this assay[15,16]

Crude yeast cell extracts were unable to substitute for RF-A in the unwinding assay An unwinding activity, however, could be detected in one fraction after chromatography of the extract on a ssDNA cellulose column The active fraction bound tightly to ssDNA cellulose, requiring 1 5 M NaCl and 50% ethylene glycol for elution, similar to the conditions that elute human RF-A The activity was further purified and found to co-fractionate on a Mono Q column with three polypeptides of relative molecular mass (M_r) 69,000 (69K), 36K and 13K (p69, p36 and p13, Fig 1*a* and *b*) The M_r of these polypeptides closely

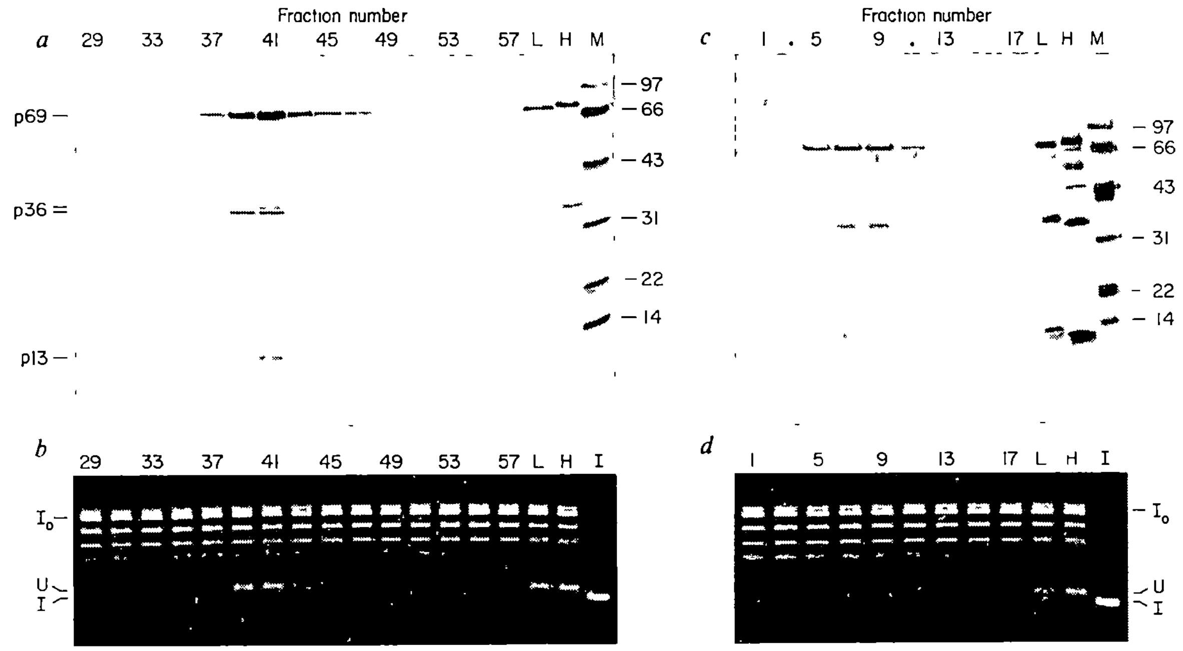

FIG 1 Co-fractionation of unwinding activity and yRF-A. *a*, SDS-PAGE (17%) and Coomassie-brilliant-blue-stain analysis of yRF-A fractions from a Mono Q column. The fractions shown represent the portion of the elution gradient from 115 to 360 mM NaCl. *b*, Unwinding assays across Mono Q. *c*, SDS-PAGE (17%) and silver-stain analysis of fractions from a glycerol gradient sedimentation of yRF-A. The fractions shown represent the entire gradient (18 fractions total). *d*, Unwinding assays across the glycerol gradient. Lanes L, load of each fractionation step, H, sample of human RF-A[10], M, relative molecular mass markers ($M_r \times 10^{-3}$). I_0, form I relaxed DNA, U, form U unwound DNA, I, form I DNA.

METHODS. Strain BJ926 (10 litres) were grown and lysed as previously described[18] except that the cells (160 g) were resuspended in 340 ml buffer A (25 mM Tris, pH 7.5, 1 mM EDTA, 10% glycerol, 1 mM DTT, 0.1 mM phenylmethylsulphonyl fluoride) with protease inhibitors (10 mM benzamidine, 100 μg ml^{-1} bacitracin, 1 μg ml^{-1} pepstatin A, 10 mM sodium bisulphite) and 1 M NaCl. The lysate was adjusted to 1 M NaCl, kept at 0 °C for 30 min, and centrifuged in a GSA rotor at 10,000 r.p.m. (16,200g at r_{max}) for 45 min. The supernatant (8.8 g protein) was adjusted to 0.5 M NaCl with buffer A and applied to a 2.5×8-cm Affi-gel Blue column. The column was washed with two volumes each of buffer A containing 0.5 M NaCl, 0.8 M NaCl and 2.5 M NaCl with 40% ethylene glycol. Peak protein fractions from the 2.5 M NaCl-ethylene glycol wash were pooled (350 mg protein), diluted to 0.5 M NaCl with buffer A and applied to a 2.5×6-cm ssDNA cellulose (USBC) column. This column was washed with two volumes each of buffer A containing 0.5 M NaCl, 0.75 M NaCl, and 1.5 M NaCl with 50% ethylene glycol. Peak protein fractions from the 1.5 M NaCl-ethylene glycol wash were pooled and dialysed against buffer A containing 200 mM NaCl and 20% sucrose. After clarification, this material (2.3 mg protein) was diluted to 100 mM NaCl with buffer A and applied to a Mono Q column (HR5/5). The column was washed with 4 ml buffer A containing 100 mM NaCl and eluted with a 10 ml linear gradient from 0.1 to 0.4 M NaCl. Fractions from this gradient (180–280 mM NaCl, peak, 220 mM NaCl) were pooled and dialysed against buffer A containing 25 mM NaCl and 20% sucrose. This procedure yielded 300 μg yRF-A at greater than 95% purity. Unwinding assays were performed as previously described[5]. Yeast RF-A (40 μg), Mono Q pool was adjusted to 0.1 M NaCl and separated on a 5-ml, 15–35% glyerol gradient in buffer A with 0.1 M NaCl (SW50.1 rotor, 49,000 r.p.m. (300,000g at r_{max}), 21 h)[4].

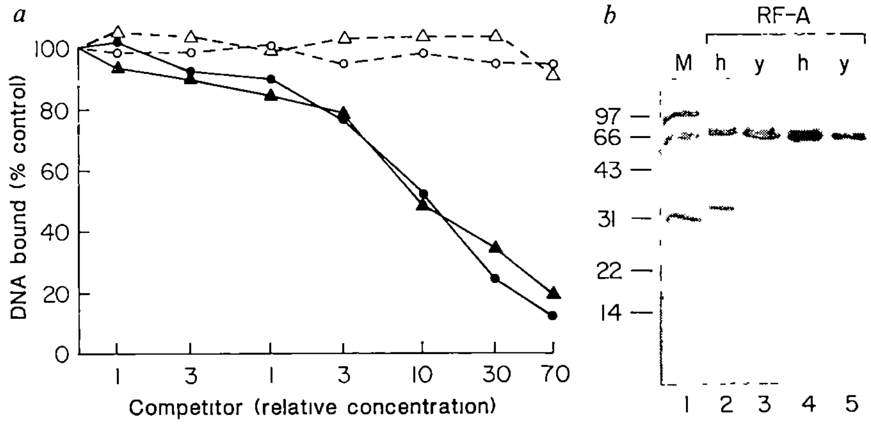

FIG 2 Single-strand DNA-binding activity of yRF-A. *a*, Competition of yRF-A binding to labelled ssDNA with double and single strand DNA. Human RF-A (triangles) or yRF-A (circles) was incubated with denatured ^{32}P-labelled pUC118 DNA in the presence of increasing amounts of double-strand pUC118 DNA (- - -) or single-stranded pUC118 DNA (——). *b*, Protein blot probed with ^{32}P-labelled DNA. Lanes 1, marker proteins, 2, 5 μg human RF-A, 3, 5 μg yRF-A, 4, 0.5 μg human RF-A, and 5, 0.5 μg yRF-A. In lanes 1–3, the nitrocellulose blot was stained with amido black to detect transferred proteins. In lanes 4 and 5, the nitrocellulose blot was probed with ^{32}P-labelled single strand pBR322 DNA. M, M_r markers ($M_r \times 10^{-3}$), h, human, y, yeast.

METHODS. *a*, Either hRF-A[10] or yRF-A (Mono Q pool) (50 ng) were incubated for 30 min at 0 °C with various amounts of unlabelled competitor DNA and 15,000 c.p.m. (5 ng) pUC118 DNA that had been digested with *Hin*dIII, filled-in with [α-^{32}P]TTP and Klenow fragment enzyme and thermally denatured. Competitor DNA consisted of pUC118 DNA digested with *Sma*I either before (double strand) or after (single strand) thermal denaturation. After the incubation the samples were filtered through alkalai-washed nitrocellulose[19] and counted. *b*, Proteins were separated by SDS-PAGE (17%), transferred to nitrocellulose, and either stained with amido black or probed[6] with thermally denatured pBR322 DNA that had been digested with *Hpa*II and filled-in with [α-^{32}P]dCTP and [α-^{32}P]dGTP.

in the complete replication reaction, although analysis of the small amount of replication products by agarose gel electrophoresis revealed topoisomers consistent with completely replicated molecules, similar to those obtained with human RF-A (Fig 4*b*)

Although yRF-A seems to fully substitute in the unwinding reaction, the ability of yRF-A to fully substitute in the complete replication reaction could depend on an additional function of RF-A We have recently shown that hRF-A stimulates both DNA polymerases α and δ, implicating a role for human RF-A in the elongation stage of DNA replication[20] Preliminary results indicate that yRF-A does not stimulate human DNA polymerase α It is probable that yRF-A, therefore, cannot efficiently substitute in the priming and elongation stages of SV40 DNA replication, because of a requirement for a species-specific interaction between RF-A and the priming-polymerase complex

The divergence of yeast and human RF-A is confirmed by the failure of both polyclonal and monoclonal antibodies, raised against human RF-A, to recognize yRF-A More recently, polyclonal antibodies have been raised against the individual subunits of yRF-A and they fail to bind to human RF-A (unpublished observation) This situation is similar to that involving the proliferating cell nuclear antigen (PCNA), another human cell replication factor that is required for SV40 DNA replication *in vitro*[8], and its yeast analogue yPCNA PCNA, from human and yeast sources, functions as an accessory protein for DNA polymerase δ and yeast DNA polymerase III, respectively Yeast PCNA fails to cross-react with antibodies raised against the mammalian protein and although yPCNA stimulates DNA polymerase δ, it does so poorly, the level of yPCNA required is 10-fold higher than the level of human PCNA required for stimulation[17]

The combined use of a mammalian cell-free system with biochemical and genetic analysis of yeast should provide an insight into eukaryotic chromosomal DNA replication A similar approach has recently been used for understanding other complex macromolecular processes, such as transcription and protein transport in eukaryotic cells The isolation of a ssDNA-binding protein from yeast that probably functions at the initiation stage of DNA replication will be a valuable entry point for understanding the mechanism of initiation of chromosomal DNA replication Because this event is highly regulated during the eukaryotic cell cycle, the components involved in the initiation of DNA replication provide attractive targets for growth regulatory processes The identification of yRF-A as a phosphoprotein should enable a biochemical and genetic analysis of those controls ☐

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1 Li J J & Kelly T J *Proc natn Acad Sci USA* **81,** 6973-6977 (1984)
2 Stillman B W & Gluzman Y *Molec cell Biol* **5,** 2051-2060 (1985)
3 Wobbe C R Dean F Weissbach L & Hurwitz J *Proc natn Acad Sci USA* **82,** 5710-5714 (1985)
4 Tsurimoto T & Stillman B *Molec cell Biol* **9,** 609-619 (1989)
5 Tsurimoto T Fairman M P & Stillman B W *Molec cell Biol* **9,** 3839-3849 (1989)
6 Wold M S Weinberg D H Virshup D M Li J J & Kelly T J *J biol Chem* **264,** 2801 2809 (1989)
7 Ishimi Y Claude A Bullock P & Hurwitz J *J biol Chem Chem* **263,** 19723-19733 (1988)
8 Stillman B *A Rev Cell Biol* **5,** 197-245 (1989)
9 Wobbe C R *et al Proc natn Acad Sci USA* **84,** 1838-1838 (1987)
10 Fairman M P & Stillman B *EMBO J* **7,** 1211-1218 (1988)
11 Wold M S & Kelly T J *Proc natn Acad Sci USA* **85,** 2523-2527 (1988)
12 Dean F B *et al Proc natn Acad Sci USA* **84,** 16-20 (1987)
13 Dodson M Dean F B Bullock P Echols H & Hurwitz J *Science* **238,** 964-967 (1987)
14 Wold M S Li J J & Kelly T J *Proc natn Acad Sci USA* **84,** 3643-3647 (1987)
15 Dean F B *et al Proc natn Acad Sci USA* **84,** 16-20 (1987)
16 Wold M S Li J J & Kelly T J *Proc natn Acad Sci USA* **84,** 3643-3647 (1987)
17 Bauer G A & Burgers P M J *Proc natn Acad Sci USA* **85,** 7506-7510 (1988)
18 Bauer G A Heller H M & Burgers P M J *J biol Chem* **263,** 917 924 (1988)
19 McEntee K Weinstock G M & Lehman I R *Proc natn Acad Sci USA* **77,** 857 861 (1980)
20 Tsurimoto T & Stillman B *EMBO J* **8** (in the press)

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Induction of the *Escherichia coli* lactose operon selectively increases repair of its transcribed DNA strand

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NUCLEOTIDE excision repair helps to ameliorate the lethal and mutagenic consequences of DNA damage by removing helix-distorting lesions from cellular genomes[1] We have previously analysed the removal of ultraviolet-induced cyclobutane pyrimidine dimers from specific DNA sequences in mammalian cells and demonstrated that transcriptionally active genes are preferentially repaired[2–4]. Additionally, we found that in rodent and human cells only the transcribed strand of the dihydrofolate reductase gene is selectively repaired[5]. Transcription is blocked by pyrimidine dimers in template DNA[6] and the selective removal of these lesions seems to be important for cell survival after irradiation with ultraviolet light[2,7,8] To determine whether this feature of repair is common to prokaryotes and eukaryotes and better to understand its mechanism, we have investigated repair in the two separate DNA strands of the lactose operon of ultraviolet-irradiated *Escherichia coli* We find a dramatic difference in the repair of the two strands only when transcription is induced. Most dimers are removed from the transcribed strand of the induced operon within five minutes of irradiation In the non-transcribed strand, repair is significantly slower and resembles that found in both strands of the uninduced operon Thus there seems to be a mechanism that couples nucleotide excision repair and transcription

The lactose operon of *E coli* is well characterized and its transcription can be modulated[9] It contains three genes in a single transcription unit, *lacZ*, *lacY* and *lacA*, which encode β-galactosidase, galactoside permease and thiogalactoside transacetylase, respectively Synthesis of the polycistronic message is induced by β-galactosides such as lactose or isopropyl-β-D-thiogalactoside (IPTG) In the absence of inducer, initiation of transcription is very infrequent

Using our earlier methodology[5 10], we measured the removal of pyrimidine dimers from the respective DNA strands of specific restriction fragments of the *lac* operon in *E coli* K12 cells grown in the presence or absence of IPTG After irradiation with ultraviolet light, culture samples were collected immediately to determine the initial frequency of dimers, and then further samples were taken after incubating for increasing periods of time to allow repair High molecular weight DNA was purified and treated with restriction enzymes Only unreplicated DNA was examined to avoid complication by dimer-free DNA generated by replication, which would cause an overestimate of the amount of repair Two aliquots of each DNA sample were analysed, one of which was treated with T4 endonuclease V to produce a single-strand break specifically at each cyclobutane pyrimidine dimer[11] Samples were electrophoresed in parallel under denaturing conditions and the DNA transferred to a support membrane These Southern blots were then hybridized with ^{32}P-labelled strand-specific RNA probes to quantitate the full-length restriction fragments at each time point As repair occurs, fewer T4 endonuclease-sensitive sites remain in the DNA, resulting in fewer strand breaks by endonuclease and more full-length restriction fragments in the enzyme-treated sample The average number of dimers per fragment was calculated from the ratio of full-length restriction fragments in the enzyme-treated and untreated samples, using the Poisson expression

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Two restriction fragments were examined the 6 6 kilobase (kb) *Apa*I-*Sst*II fragment that contains 1 5 kb of sequence outside the coding region of the operon, and the 5 2 kb *Nar*I-*Nru*I restriction fragment that resides almost entirely within the transcription unit (Fig 1*a*) Strand-specific RNA probes (Fig 1*b*) were obtained by subcloning a fragment of the *lacZ* gene into the multiple cloning site of the vector pGEM-3Z The construct, pZH10, contains the *lacZ* sequence flanked by two different phage RNA polymerase promoters oriented in opposite directions Linearizing the plasmid with *Eco*RI and incubating with SP6 RNA polymerase generates an RNA probe specific for the transcribed strand of the operon, whereas linearizing with *Bam*HI and incubating with T7 RNA polymerase generates an RNA probe specific for the non-transcribed strand

Repair was examined in the 6 6 kb *Apa*I-*Sst*II fragment of the uninduced or induced operon at 2 5, 5, 10 and 20 min after irradiation with ultraviolet light ($40\ \mathrm{J\,m^{-2}}$) (Fig 2) Levels of β-galactosidase were 4,800 units and 11 units[12] in cultures grown in the presence or absence of IPTG Southern blots were hybridized with the probe specific for the transcribed strand, that probe was removed and the blot was then hybridized with the probe specific for the non-transcribed strand The initial level of damage was the same in each strand, whether or not transcription had been induced Visual comparison of the enzyme-treated samples probed for the two different strands at each repair time point reveals similar amounts of full-length restriction fragments when the operon was not induced, but significantly more full-length fragments for the transcribed strand when the operon was induced Quantitation indicates that there is a dramatic difference in the rate of repair in the transcribed and non-transcribed strands of the operon only when transcription was induced with IPTG (Fig 3) Within 5 min, most dimers were removed from the transcribed strand of the induced operon, whereas little repair was detected at this time in the non-transcribed stand or in either strand of the uninduced operon The repair rates in each strand of the uninduced operon and in the non-transcribed strand of the induced operon were comparable Repair was almost complete within 40 min after irradiation (data not shown)

This study provides two important observations (1) Selective removal of pyrimidine dimers from the transcribed strand of an active gene is not unique to mammalian or even eukaryotic excision repair Repair in the induced *lac* operon of ultraviolet-irradiated *E coli* resembles repair in the active dihydrofolate reductase (*DHFR*) gene of ultraviolet-irradiated human cells[5] repair in the transcribed strand is significantly faster than that in the non-transcribed strand, but most dimers are removed from both strands within one generation period in the respective cell types (2) This selective removal of pyrimidine dimers from the transcribed strand of a gene is abolished in the absence of significant levels of transcription

Bockrath and Palmer[13] studying ultraviolet-irradiated *E coli* noted a more rapid decline in mutations generated by lesions that were deduced to be in the transcribed strand of a glutamine transfer RNA gene than in mutations generated by lesions in the non-transcribed strand of the gene This difference in mutation frequency did not occur in a mutant deficient in nucleotide excision repair They suggested that selective repair of the transcribed strands of the $^{\mathrm{Gln}}$tRNA genes might account for the observed decline in mutation frequency, a notion strengthened by the demonstration that the strand-specific loss of mutations is unrelated to the polarity of replication[14] We have been unable to analyse repair in the respective strands of the $^{\mathrm{Gln}}$tRNA genes (unpublished data) because the method described here requires very high doses of ultraviolet light to examine small sequences A similar strand bias in mutation frequency was noted by Bockrath *et al*[15] using ethyl methanesulphonate, and Reed and Hutchinson[16] have reported a large bias in mutation frequency towards the non-transcribed strand of the λ *cI* gene in *E coli* treated with another alkylating agent, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine It will be important to determine whether differences in the removal of DNA damage from the respective strands of active genes in *E coli* and mammalian cells correlate

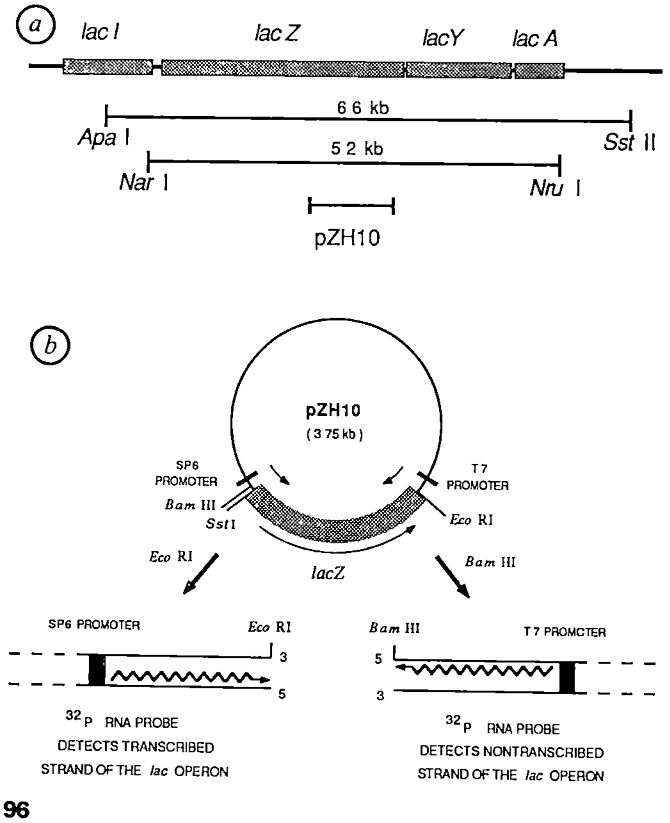


FIG 1 *a*, Map of *E coli* lactose operon The positions of the genes within the operon, *lacI*, the *lacZ* insert in pZH10 used to generate probes, and relevant restriction enzyme sites are shown *b*, Illustration of the plasmid pZH10 and application to generate strand-specific RNA probes Plasmid pZH10 contains 1 kb of the *E coli lacZ* gene (thick arc) and the vector sequences from pGEM-3Z (thin line), which include the SP6 and T7 RNA polymerase promoters (heavy bars) The directions of transcription of the *lacZ* gene *in vivo* and the SP6 and T7 promoters are indicated by arrows around the plasmid diagram Strand-specific RNA probes are indicated by wavy lines

METHODS Restriction enzyme sites in the *lac* operon region were obtained from sequence data (accession numbers J01636, J01637, K01483, K01793) in the GenBank Data Bank[27] (release 59) using the Bionet resources (Intelligenetics) The plasmid pZH10 was constructed by isolating a 1 kb *Eco*RI-*Sst*I *lacZ* fragment from the plasmid pZH1 (ref 28) and subcloning it into the *Eco*RI-*Sst*I sites of pGEM-3Z Cultures of DH5alpha (BRL) were transformed with the ligation products, and recombinants were selected as white colonies on LB plates supplemented with $100\ \mu\mathrm{g\,ml^{-1}}$ ampicillin, 0 5 mM IPTG, and $40\ \mu\mathrm{g\,ml^{-1}}$ X-Gal Plasmids were isolated and purified by two consecutive bandings in CsCl/ethidium bromide gradients Plasmid DNA was digested with *Bam*HI (*Sst*I generates 3′ protruding ends) or *Eco*RI and RNA probes were prepared as previously described[5]

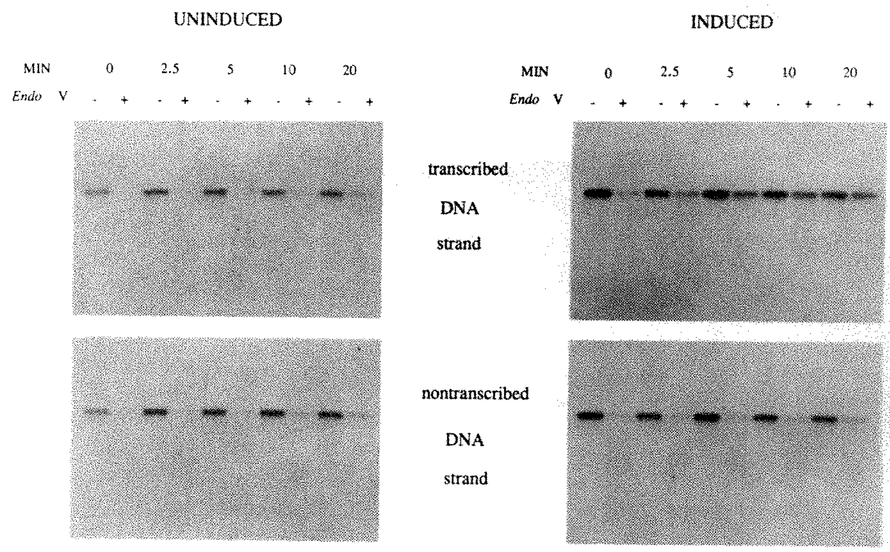

UNINDUCED
MIN 0 2.5 5 10 20
Endo V - + - + - + - + - +
INDUCED
MIN 0 2.5 5 10 20
Endo V - + - + - + - + - +
transcribed DNA strand
nontranscribed DNA strand

FIG. 2 Autoradiograph illustrating repair in the *lac* operon when it is uninduced and induced. DNA was isolated at the times indicated above the lanes, restricted with *Apa*I and *Sst*II, and not treated (−) or treated (+) with T4 endonuclease V. Samples (0.5 μg) of DNA were loaded. ^{32}P-labelled RNA probes were made from pZH10.

METHODS. Cells were grown, irradiated with ultraviolet light, and incubated as previously described[29], with some modifications. Cultures of *E. coli* K-12 strain SR108 ($F^{-}\lambda^{s}$*thyA deo*$^{-}$) (provided by A. Ganesan) were grown at 37 °C in Difco Bacto Minimal Broth Davis, supplemented with 0.4% glucose, 0.05% casein hydrolysate, $2\ \mu g\ ml^{-1}$ thymine and $0.5\ \mu Ci\ ml^{-1}$ [^{3}H]thymine. Cultures were grown to a density of $\sim 3 \times 10^{7}$ cells per ml, collected on 0.22-μm pore size Millipore filters (GSWP) by filtration, and resuspended at the original density in unsupplemented Minimal Broth. Cultures were irradiated with 40 J per m^{2} of ultraviolet light (254 nm) and incubated for the specified repair periods in Minimal Broth containing 0.4% glucose, 0.05% casein hydrolysate and $10\ \mu g\ ml^{-1}$ 5-bromouracil. For studies of repair in the induced *lac* operon, IPTG (0.1 mM) was included in the medium before and after ultraviolet radiation. (Because glucose was present in the medium, the operon was not fully induced.) β-Galactosidase levels were assayed as previously described[12], except cells were permeabilized by freeze-thawing instead of by toluene treatment. High molecular weight DNA was isolated[29], treated with restriction enzymes, sedimented to equilibrium in CsCl density gradients to remove replicated DNA and treated with T4 endonuclease V (ref. 5). Samples of DNA (0.5 μg) were treated with 1 μl T4 endonuclease V (incision activity of enzyme preparation, 5.5×10^{11} sites per $\mu l\ min^{-1}$ at 37 °C). Samples were electrophoresed in 0.6% alkaline agarose gels and the DNA was transferred to a Genatran nylon membrane. The membrane was hybridized with ^{32}P-labelled probe, washed and exposed to X-ray film as described[5]. The amount of radioactivity hybridized to the fragment of interest was determined by scanning densitometry of the autoradiograph.

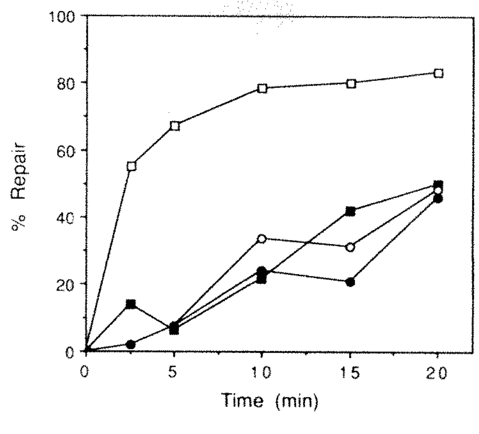
with mutation frequencies in these strands, but this examination may be complicated by other cellular processes that influence mutation frequency[17-19].

There is strong evidence that transcription is blocked by pyrimidine dimers in template DNA in both prokaryotic and eukaryotic cells[6]. Initiation occurs normally on the damaged template (assuming no damage in the initiation region) and chain elongation proceeds until a dimer is encountered, resulting in premature termination. It is likely that only damage to the template strand produces this effect; this has been directly shown *in vitro* for psoralen monoadducts[20]. A repair mechanism that specifically detects transcription-blocking DNA damage should be advantageous to the cell if maintenance of constant levels of essential transcripts is critical for cell survival. As ultraviolet irradiation actually induces the transcription of a collection of genes[21] while producing DNA lesions that block transcription elongation, the rapid removal of transcription-blocking lesions should facilitate the expression of such ultraviolet-induced genes. It is tempting to speculate that for some genes, the purpose of ultraviolet-induced transcription is not simply to provide a gene product, but rather to ensure that DNA damage is rapidly removed from certain essential sequences.

The molecular details of nucleotide excision repair are more complicated and less well understood in mammalian cells than in *E. coli*. A larger number of genes are involved and although some have recently been cloned[22,23], their functions are unknown. It is also not clear how heterogeneities in the chromatin structure or organization of particular sequences in the complex mammalian genome affect DNA repair. Indeed, we originally suggested that differences in the repair of transcriptionally active and inactive sequences in mammalian cells are due to differences in their chromatin structure[2,4]. Our finding that preferential repair of the *DHFR* gene in human and rodent cells is due to selective repair of only the transcribed strand of the gene challenged this model. The strand-specific repair in the *E. coli lac* operon indicates that features of eukaryotic chromatin structure are not required for selective removal of damage from active genes. Furthermore, as this feature of repair has been conserved in bacteria and mammalian cells, it is not something that just evolved in higher eukaryotes as a mechanism to deal

FIG. 3 The effect of transcription on pyrimidine dimer removal from the DNA strands of the *lac* operon. The average number of dimers was measured in the 6.6-kb *Apa*I-*Sst*II restriction fragment at 0, 2.5, 5, 10 and 20 min after irradiation (40 J m^{-2}) and in the 5.2-kb *Nar*I-*Nru*I restriction fragment at 0, 5, 10, 15 and 20 min after irradiation (40 J m^{-2}). Values at 5, 10 and 20 min are an average of results from the two fragments. T (open symbols), transcribed strand; NT (filled symbols), non-transcribed strand; squares, induced; circles, uninduced.

METHODS. The average number of dimers per fragment was calculated from the percentage of fragments that had no dimers ('zero class') by using the Poisson expression (number of dimers per fragment = $-\log_e$ of the zero class). The zero class is equal to the ratio of the amount of full-length fragments in the enzyme-treated and untreated samples.



with the organization of sequences within their complex genomes.

A comprehensive description of nucleotide excision repair has only been achieved for *E. coli*[24,25]. Most, if not all, of the genes involved have been cloned and their products studied *in vitro*. At least six proteins are involved: UvrA, UvrB and UvrC, assembled in an 'excinuclease complex', and UvrD, DNA polymerase I and DNA ligase. It is thought that the distortion of the DNA helix produced by the pyrimidine dimer (or bulky DNA adducts), rather than the damage itself, is the substrate for the UvrABC complex. Two incisions are made in the DNA strand near the damage: the eighth phosphodiester bond 5′ to, and the fourth or fifth phosphodiester 3′ to a pyrimidine dimer are cleaved. The oligonucleotide containing the dimer is removed, a repair patch is synthesized using the complementary strand as a template, and ultimately the repair patch is ligated to the contiguous parental DNA strand. The strand-specificity in the repair of the induced *lac* operon demonstrated here indicates an association or coupling between transcription and nucleotide excision repair. Considering the ultraviolet-sensitive mutants that have been characterized in *E. coli*, it seems unlikely that the preferential repair of the transcribed strands of active genes is carried out by an entirely different and as-yet-uncharacterized repair system. It is likely that selectivity is imposed by targeting of the UvrABC complex to damage in the transcribed strand. There need not be a specific factor whose sole role is to mediate such targeting. For example, an RNA polymerase complex blocked at a lesion in the transcribed strand may itself facilitate recognition. This could be analogous to the mechanism whereby *E. coli* photolyase, an enzyme that specifically binds and photo-reverses pyrimidine dimers, actually stimulates the removal of dimers by the UvrABC excision nuclease[26]. Studies using the genetic and biochemical tools available in *E. coli* are needed to test this model, which should enhance our understanding of transcription-associated DNA repair in both bacteria and mammalian cells. ☐

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1. Friedberg, E. C. in *DNA Repair* (Freeman, San Francisco, 1985).
2. Bohr, V. A., Smith, C. A., Okumoto, D. S. & Hanawalt, P. C. *Cell* **40**, 359–369 (1985).
3. Madhani, H. D., Bohr, V. A. & Hanawalt, P. C. *Cell* **45**, 417–423 (1986).
4. Mellon, I., Bohr, V. A., Smith, C. A. & Hanawalt, P. C. *Proc. natn. Acad. Sci. U.S.A.* **83**, 8878–8882 (1986).
5. Mellon, I., Spivak, G. & Hanawalt, P. C. *Cell* **51**, 241–249 (1988).
6. Sauerbier, W. & Hercules, K. *A. Rev. Genet.* **12**, 329–363 (1978).
7. Mayne, L. V. & Lehmann, A. R. *Cancer Res.* **42**, 1493–1498 (1982).
8. Mayne, L. V., Mullenders, L. H. F. & van Zeeland, A. A. in *Mechanisms and Consequences of DNA Damage Processing* (eds Friedberg, E. C. & Hanawalt, P. C.) 349–353 (Liss, New York, 1988).
9. *The Lactose Operon* (Cold Spring Harbor Laboratory, New York, 1970).
10. Bohr, V. A. & Okumoto, D. S. in *DNA Repair: A Laboratory Manual of Research Procedures* (eds Friedberg, E. C. & Hanawalt, P. C.) **3**, 347–366 (Dekker, New York, 1988).
11. Sancar, A. & Rupp, W. D. *Cell* **33**, 249–260 (1983).
12. Miller, J. H. in *Experiments in Molecular Genetics* 352–355 (Cold Spring Harbor Laboratory, Cold Spring Harbor, 1972).
13. Bockrath, R. C. & Palmer, J. E. *Molec. gen. Genet.* **156**, 133–140 (1977).
14. Engstrom, J., Larsen, S., Rogers, S. & Bockrath, R. C. *Mutation Res.* **132**, 143–152 (1984).
15. Bockrath, R. C., Barlow, A. & Engstrom, J. *Mutation Res.* **183**, 241–247 (1987).
16. Reed, J. & Hutchinson, F. *Molec. gen. Genet.* **208**, 446–449 (1987).
17. Vrieling, H. *et al. Molec. cell. Biol.* **9**, 1277–1283 (1989).
18. Herman, R. K. & Dworkin, N. B. *J. Bact.* **106**, 543–550 (1971).
19. Savić, D. J. & Kanazir, D. T. *Molec. gen. Genet.* **118**, 45–50 (1972).
20. Shi, Y. B., Gamper, H. & Hearst, J. E. *Nucleic Acids Res.* **15**, 6843–6854 (1987).
21. Fornace, A. J., Jr, Alamo, I., Jr, & Hollander, M. C. *Proc. natn. Acad. Sci. U.S.A.* **85**, 8800–8804 (1988).
22. Hoeijmakers, J. H. J. *et al.* in *Mechanisms and Consequences of DNA Damage Processing* (eds Friedberg, E. C. & Hanawalt, P. C.) 281–287 (Liss, New York, 1988).
23. Thompson, L. H., Weber, C. A. & Carrano, A. V. in *Mechanisms and Consequences of DNA Damage Processing* (eds Friedberg, E. C. & Hanawalt, P. C.) 289–293 (Liss, New York, 1988).
24. Sancar, A. & Sancar, G. W. *A. Rev. Biochem.* **57**, 29–67 (1988).
25. Grossman, L., Caron, P. R., Mazur, S. J. & Oh, E. Y. *Fedn Proc.* **2**, 2696–2701 (1988).
26. Sancar, A., Franklin, K. A. & Sancar, G. B. *Proc. natn. Acad. Sci. U.S.A.* **81**, 7397–7401 (1984).
27. Bilofsky, H. S. *et al. Nucleic Acids Res.* **14**, 1–4 (1986).
28. Ganesan, A. K. & Spivak, G. in *DNA Repair: A Laboratory Manual of Research Procedures* (eds Friedberg, E. C. & Hanawalt, P. C.) **3**, 295–310 (Dekker, New York, 1988).
29. Smith, C. A., Cooper, P. K. & Hanawalt, P. C. in *DNA Repair: A Laboratory Manual of Research Procedures* (eds Friedberg, E. C. & Hanawalt, P. C.) **18**, 289–305 (Dekker, New York, 1981).

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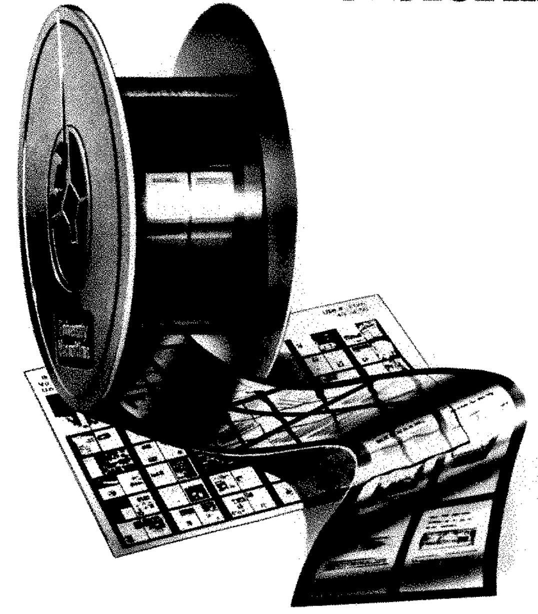

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Engineering monoclonal antibodies

J.D. Rodwell

Efforts to alter the mAb component of pharmaceuticals have focused on making them more human-like and smaller. Ultimately, the smallest molecular recognition unit derived from antibodies may be a single complementarity region.

MURINE monoclonal antibodies (mAbs) conjugated to drugs, isotopes, or toxins are being utilized as the targeting components of diagnostic and therapeutic agents for cancer, cardiovascular and other diseases. However, because of the limitations of administering nonhuman proteins, efforts to engineer new generations of antibodies, or antibody-like molecules, are already underway in both the biotechnology industry and in academe. These efforts are directed towards making the antibodies more human-like, smaller in size, or both (Fig. 1), with the aim of lowering the immunogenicity of these proteins in man and optimizing their target penetration and pharmacokinetic characteristics.

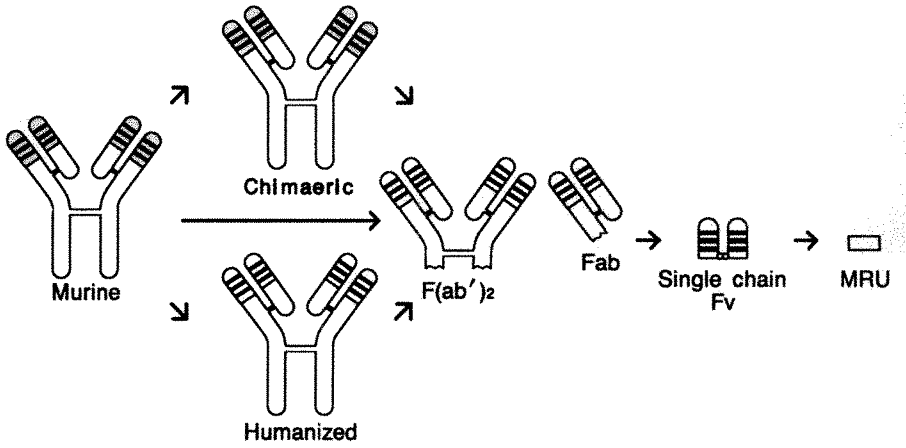

FIG. 1 Efforts to alter the mAb component of pharmaceuticals have led to the development of chimaeric, CDR-grafted (humanized) antibodies and to progressively smaller molecules.

mAb immunogenicity

One way to decrease mAb immunogenicity, as described by Morrison and her colleagues[1], involves the development of 'chimaeric' antibody technology. Rearranged DNA encoding mouse variable (VH, VL) regions is fused to DNA encoding human constant heavy and light chains. On expression, interspecies antibody chimaeras are obtained, with the same antigen-binding specificity as the parent molecule. Because most of the protein comprises human amino acid sequences, these antibodies should be less antigenic. A recent report by LoBuglio *et al.*[2] suggests that this approach may, indeed, result in reduced immunogenicity. A chimaeric antibody derived from the mouse monoclonal 17-1A was administered to patients with metastatic colon cancer. Compared to the parent murine antibody, the chimaeric molecule was substantially less immunogenic, with only one in 10 patients developing an antibody response.

Another approach is CDR (complementarity determining regions) grafting, which was developed by Winter and his colleagues[3]. This technique involves incorporation of the CDRs of mAbs into an expression system encoding human variable and constant regions. As the resulting 'humanized' antibodies contain only a small fraction of the amino acid sequence of mouse origin, it was reasoned that there would be little problem with immunogenicity. In a preliminary communication[4], Hale *et al.* reported remission in two non-Hodgkin lymphoma patients who received daily doses of 1–20 mg of a humanized mAb (CAMPATH-1-H) over 43 days. Significantly, neither patient developed a detectable immune response to the antibody.

Molecular size

While the above approaches may reduce patient sensitization to injected antibodies, they do not deal with the problem of molecular size. One potential solution is the use of antigen-binding fragments (Fabs), which penetrate target tissue better and clear from blood more rapidly than intact antibodies. As both chimaeric and humanized antibodies can be substrates for Fab production, Fabs produced in this way offer the same advantages over intact antibodies as mouse Fabs, while retaining lower immunogenicity. For diagnostic imaging of highly vascularized regions, such as thrombi, background clearance of labelled Fabs through the kidneys allows more rapid visualization of damaged myocardium. For tumour therapy, where the goal is to deliver a cytocidal dose of an appropriate isotope, drug, or toxin to as many target cells as possible, Fabs penetrate tumours more effectively, producing a more even distribution[6]. The cytotoxic efficacy of therapeutic isotopes may be expected to rise with better and more uniform tumour penetration.

The underlying assumption that smaller molecular size is better has also been addressed by the preparation of antigen-binding Fv fragments and 'single-chain' antibodies. Fvs are specific variable regions pairs (VH and VL), and have been expressed in *Escherichia coli*[6] and myeloma cells[7]. Single-chain antibodies are an interesting variation on this approach, in that VH and VL genes are joined together by a DNA segment encoding a peptide. This peptide is capable of bridging the carboxy terminus of one V domain and the amino terminus of the other, resulting in an Fv-like molecule synthesized as one continuous amino acid polymer. This approach has been reported to yield a properly folded (and paired) VH-VL gene product with the same antigen-binding characteristics as the antibody from which it was derived[8,9].

Future directions

All of these new approaches promise to improve upon results achieved with intact murine mAbs, and so we are developing strategies for the clinical evaluation of each approach. However, simplification of the molecular recognition component of this new generation of pharmaceuticals will not end here. At least two independent groups have reported that peptides, each representing the sequence of a single CDR from a mAb, can mimic the specificity of the parent molecule.

Williams *et al.*[10] have shown that a peptide with the sequence of the second CDR of the light chain of mAb 87.92.6, which binds to the reovirus type-3 receptor on cells, mimics the ability of the antibody to 'down-regulate' the receptor and inhibit DNA synthesis. Taub *et al.*[11] have described a peptide with the sequence of the third CDR of the heavy chain of PAC1 — an antibody specific for the GP IIb-IIIa complex on activated human platelets. Like the parent antibody, this peptide inhibits the binding of fibrinogen to platelets and also inhibits the binding of the PAC1 antibody itself. In both cases, the CDR peptides have homology with the sequence of the 'native' protein ligand.

Williams' peptide has sequence homology with the reovirus 3 haemagglutinin, and Taub's peptide has sequence homology with a region of fibrinogen that binds to the GP IIb-IIIa complex.

The CDRs of antibodies may, therefore, be an important source of amino-acid seqüence information that would facilitate the preparation of small recognition elements. The molecular weights of these recognition elements would be as low as a few per cent of the weight of an IgG antibody. Consequently, these elements, conjugated or fused to diagnostic and therapeutic effector molecules, would constitute an important new class of self-targeting pharmaceuticals.

The clinical potential for the diagnosis and treatment of human diseases with self-targeting pharmaceuticals using mouse mAbs is finally being realized. Knowledge derived from the use of intact antibody conjugates will allow further innovation as each component (molecular recognition, effector) is better understood. We can expect new generation(s) of antibody-like recognition units to be engineered using molecular surgery to remove structures that have evolved for functions secondary to antigen binding. □

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1. Morrison, S.L., Johnson, M.J., Herzenberg, L.A. & Oi, V.T. *Proc. natn. Acad. Sci. USA* **81**, 6851–6855 (1984).
2. LoBuglio, A.F. *et al. Proc. natn. Acad. Sci. USA* **86**, 4220–4224 (1989).
3. Jones, P.T., Dear, P.H., Foote, J., Neuberger, M.S. & Winter, G. *Nature* **321**, 522–525 (1986).
4. Hale, G. *et al. Lancet* **II 8625**, 1394–1399 (1988).
5. Buchegger, F. *et al. J exp. Med.* **158**, 413–427 (1983).
6. Skerra, A. & Pluckthun, A. *Science* **240**, 1038–1041 (1988).
7. Riechmann, L. & Foote, J. *J. molec. Biol.* **203**, 825–828 (1988).
8. Huston, J.S. *et al. Proc. natn. Acad. Sci. USA* **85**, 5879–5883 (1988).
9. Bird, R.E. *et al. Science* **242**, 423–426 (1988).
10. Williams, W.V. *et al. Proc. natn. Acad. Sci. USA* **86**, 5537–5541 (1989).
11. Taub, R. *et al. J. biol. Chem.* **264**, 259–265 (1989).

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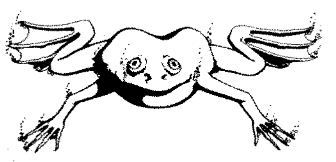

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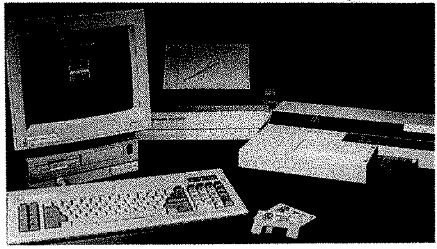
Biology's soft cell

Highlights at next week's American Society for Cell Biology Annual Meeting in Houston, Texas include an interactive 3-D graphics software package and formulin-free tissue fixative.

FLOW Laboratories, exhibiting in booth 1241, has two new **PC-based microplate readers**, designed to scan a 96-well plate in less than two seconds (*Reader Service No. 101*). The £5,495 (UK) Multiskan Plus MKII and £6,950 (UK) Multiskan MCC/340 MKII PC systems come complete with microplate reader, IBM PC/XT or compatible, monochrome monitor, data capture and analysis software and 80-column printer. Switching assay routines is as simple as changing the disk, says Flow. The standard curve and sample results can be viewed via the monitor or printed as hard copy. The Entry Level Computing software provides statistical analyses, including mean minimum, maximum, delta, standard deviation, delta percentage and coefficient of variance for each row, column and whole plate. In addition to the five measurement modes, and nine calculation modes, the more advanced Multiskan MCC/340 MKII has a multiwavelength mode and an eight filter wheel for automatic dual wavelength reading.



Microplate reading IBM-compatibly.

In a fix

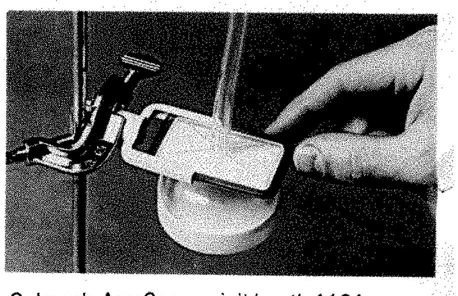
Zymed Laboratories are offering — OmniFix — **formulin-free tissue fixative** (*Reader Service No. 102*). This alcohol-based, odourless tissue fixative is, says the company, less toxic than formaldehyde and can be used with common staining techniques, such as peroxidase anti-peroxidase, avidin-biotin complex and streptavidin-peroxidase. According to Zymed, fixation times are reduced as OmniFix penetrates tissues more readily than formulin. Zymed recommends that tissues should be immersed for at least two hours. OmniFix is supplied ready-to-use in 1-, 5-, and 55-gallon polyethylene containers, and samples are available on request. Zymed will be in booth 1215.

According to Vector Laboratories, their Vectabond **tissue section adhesive** can significantly increase the adherence of both frozen and paraffin-embedded tissue sections to glass slides (*Reader Service No. 103*). Vectabond chemically modifies the glass, says Vector, to form an adherent surface that is suitable for all types of tissue sections and *in situ* hybridizations, while avoiding the instability and protease susceptibility problems associated with conventional adhesives. Vectabond costs $35 (US) for a 7-ml concentrate that makes 350 ml of treatment solution — enough for at least 500 slides, says Vector, who will be exhibiting in booth 749.

Culture conveniences

According to Becton Dickinson Labware, increased cell production is possible with their new line of Falcon brand **expanded surface roller bottles** (*Reader Service No. 104*). The longitudinal pleat design of the roller bottles improves cell yields and reduces the amount of trypsin required for cell harvesting, says the company. Other design features of these high-grade polystyrene roller bottles include a viewing side panel and a patented plug seal cap that reduces the risk of leakage and contamination, says Becton Dickinson. The Falcon brand roller bottles are sold in cases of 20 bottles for $144 (US). The company will be exhibiting in booths 1501/1503.

Using Gelman Sciences' AcroCap **presterilized disposable filters**, the company says serum-free culture media, media additives and buffers can be filtered at flow rates of 200 ml per minute (*Reader Service No. 105*). The AcroCap filters incorporate 15 cm^2 of Supor membrane, which is designed to filter up to 3 litres. The filling-bell attachment fits over wide-mouth cell culture bottles and, says Gelman, reduces the risk of contamination when filtering directly into sterile flasks. A 0.02 μm PTFE vent is incorporated into the filter's design to help prevent air blockage. The filters can attach to standard 0.25-inch tubing and can be used in conjunction with a peristaltic pump or repeating syringe. AcroCap filters come in 0.2 μm and 0.45 μm pore sizes and



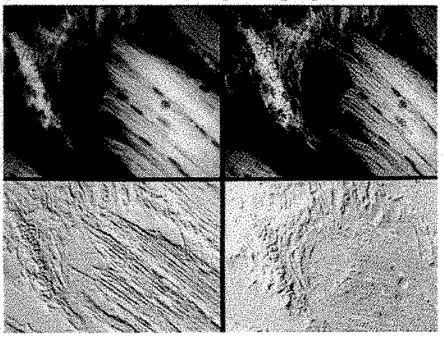
Gelman's AcroCap — visit booth 1101.

cost $35 (US) for 10 filters. Gelman Sciences will be in booth 1101.

The right image

Visitors to booth 1042, can see VoxelView — an **interactive volume rendering software system** — from Vital Images, Inc., which allows the user to visualize and manipulate 3-dimensional data sets in real time (*Reader Service No. 106*). VoxelView graphically reconstructs objects from 3-D voxel building blocks without, says Vital Images, any loss of complexity or resolution, and at speeds of up to 4 million voxels per second. The system can render data from sources as diverse as confocal microscopes, medical imaging systems, histological sections and molecular models. Using interactive pop-up menus, a pictorial data catalogue and graphical control of rendering parameters, the user can adjust image contrast/magnification, assign pseudocolour to images, minimize artefacts, adjust lighting and transparency, rotate and view data from any angle. Objects or phenomena deemed to be short-lived can, says Vital Images, be saved as 3-D computer images. VoxelView is designed to run on any Silicon Graphics 4D/GT or GTX series workstation, and is delivered as a 0.25-inch tape cartridge complete with user's guide.

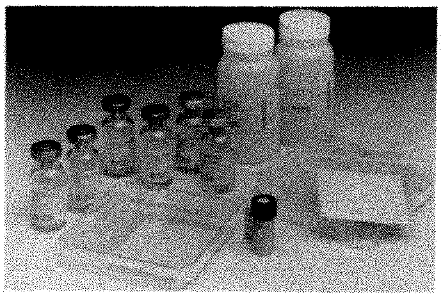
Universal Imaging Corporation, exhibiting in booths 128, 130 and 132, will be demonstrating the Image-1/AT combined **image processing and analysis system** (*Reader Service No. 107*). The $16,900 (US) Image-1/AT Combined System runs on IBM AT-compatible computers and has menu-driven software. The video-processing board allows video-rate image processing and high-speed graphics. Other functions include real-time image averaging with background subtraction, object counting and sizing, image sharpening, zoom and roam, 3-D image generation from optical sections, gel scanning and densitometry, text and graphics overlays. Images can be stored on magnetic disk or on an optional optical memory disk recorder. Universal emphasizes the system's built-in flexibility in that users can produce custom menus and journals — user-defined sequences of commands for repeat operations —which eliminate the need for laborious programming and which can be recalled by a single keystroke command.



Video viewing, courtesy Universal Images.

Assays, etc.

Advanced Magnetics, Inc are offering two new **immunoblot assay kits** for the measurement of phosphatidylinositol-4-monophosphate (PIP) and phosphatidylinositol 4,5-bisphosphate (PIP_2) (*Reader Service No. 108*). Samples and standards are bound to a nitrocellulose membrane and incubated overnight with monoclonal PIP antibody. The membrane is washed and incubated with goat anti-mouse IgM-HRP. The HRP substrate is developed with diaminobenzidine. Sample concentrations are determined by performing a colour comparison of sample and standard dots on the membrane. Each $395 (US) PIP and PIP_2 kit comes with sufficient reagents for 100 tests. AMI will be in booth 319.



Phosphatidylinositol phosphate kits.

Promega Corporation, exhibiting in booths 414/416, has **CAT reporter systems** for the direct assay of gene regulatory elements by measuring chloramphenicol acetyltransferase (CAT) activity (*Reader Service No. 109*). Genomic DNA fragments containing putative regulatory sequences are cloned into specially-constructed plasmids containing the CAT reporter gene. Four $55 (US) pCAT plasmids are available — pCAT Basic, pCAT Enhancer, pCAT Promoter and pCAT Control — all of which share a common pUC19 backbone and express CAT activity in eukaryotic cells. Promega's CAT reporter system offers two methods for monitoring CAT enzyme activity in transfected cells: liquid scintillation counting or thin-layer chromatography. The LSC assay takes 2-3 hours and can detect 3×10^4 units (2 pg) of CAT, says Promega. The TLC assay takes longer, is less sensitive, but provides visual confirmation of assay results by autoradiography. The CAT enzyme assay, which includes 100 units chloramphenicol acetyltransferase, 25 ml Tris-HCl and 255μl *n*-butyryl-CoA, costs $75 (US). ☐

These notes are compiled by Diane Gershon from information provided by the manufacturer. To obtain additional information about these products, use the reader service card bound inside the journal. Prices quoted are sometimes nominal, and apply only within the country indicated.

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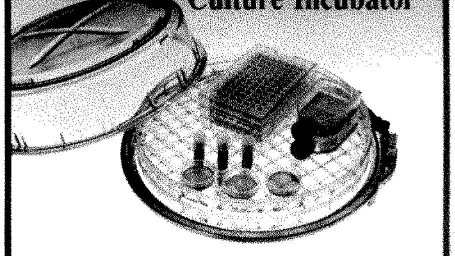

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MUNICH – Sabine Fürst, Sandstrasse 41 8000 München 2. Telephone: (089) 52 40 82. Fax: (089) 5 23 22 22.
TOKYO – Phillip Hamill, Shin-Mitsuke Building (4F), 3–6 Ichigaya Tamachi, Shinjuku-ku, Tokyo 162. Telephone: (03) 267 8751. Fax: (03) 267 8746.

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LINCOLN COLLEGE

(University from 1990)

New Zealand

PROFESSOR OF SOIL SCIENCE

The Council of Lincoln College invites applications from suitably qualified men and women for the Chair of Soil Science. The Chair is one of 16 at the College and the Council is anxious to fill the position with an appointee of high standing because of its significance, not only to the College, but also to New Zealand.

Applicants should hold an advanced degree in an appropriate discipline of Science and have an established record of research and teaching in Soil Science. Applicants should have a proven interest in the wider role of soils in the environment, with a competence in any of the major branches of Soil Science. The appointee will be expected to foster and develop the teaching, research and extension activities of the Department as a whole and to contribute to the research programme in his/her own field.

It is anticipated that the successful applicant will be appointed as Head of Department for an initial period of three years and will be responsible to the Principal for the administration of the Department.

Lincoln College will be granted autonomous University status in 1990. The 50 ha campus is 21 km south of the City of Christchurch. There are approximately 2000 students on Campus. Christchurch is the third largest city in New Zealand, the largest in the South Island, with a population of approximately 300,000 people and is served by direct international flights from Australia, Asia, United States of America and the United Kingdom.

Salary will be paid at a step in the range of NZ$77,000 – NZ$96,000 per annum. Salaries are reviewed annually. Expenses incurred in taking up the appointment will be reimbursed up to specified limits. Government Superannuation is available.

Further information about the position including details of the structure and academic functions of the Department, application procedure, travel and removal expenses, housing assistance and conditions of appointment are available from **Appointments (37081) Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF, UK, or from A.J. Sargison, Registrar, PO Box 94, Lincoln College, Canterbury, New Zealand, Fax No. (64)(3) 252-965,** with whom applications quoting vacancy no 89/51 close on **15 January 1990.**

Lincoln College is an equal opportunity employer (W6665)A

UNIVERSITY OF ABERDEEN

DEPARTMENT OF ANATOMY

LECTURER

Applications are invited for the above post, tenable for five years. The appointee will be expected to attract external funding for and to conduct active quality research in the preferred areas of Cell Biology, Neurobiology or Developmental Biology, to assist in teaching gross anatomy to medical students and to participate in and develop modular degree courses for science students. The Department has research facilities for light and electron microscopy, stereology, computer-assisted image analysis and cell/tissue culture.

Initial salary will be on the Lecturer scale £10,458-£20,469 per annum with appropriate placing.

Further particulars and application forms from the **Personnel Office, The University, Regent Walk, Aberdeen AB9 1FX (tel 0224 273500),** to whom applications (2 copies) should be returned by 24 November 1989 quoting reference number JA/070. (1977)A

Mervin Bovaird Center in Molecular Biology and Biotechnology

■ ■ ■

MICROBIOLOGIST

MOLECULAR BIOLOGIST/VIROLOGIST

MOLECULAR IMMUNOLOGIST

TRANSGENIC MOUSE EXPERT

Due to major funding acquisitions through **The Mervin Bovaird Foundation** and a cooperative **Center of Excellence in Molecular Medicine**. The University of Tulsa is expanding its tenure-track faculty.

Researchers will be provided renovated laboratory space, funded post-doctoral positions, and generous start-up monies. **The transgenic mouse facility is guaranteed five years of substantial funding including technician in addition to start-up monies**. Requirements: Ph.D., postdoctoral experience, publication record indicative of the potential to maintain an externally funded research program. Salary and rank commensurate with experience. Faculty are expected to teach undergraduate and graduate students, and to maintain a record of productivity in scholarly research.

The department has sixteen faculty and offers BS, MS, and Ph.D. degrees. Extramural funding in 1987-89 totaled more than $5 million. Facilities include state-of-the-art laboratories and equipment.

The university is a private, heavily endowed, comprehensive university of four thousand students with a strong commitment to teaching and research. Tulsa is an economic and cultural center of 750,000 surrounded by lakes. Applicants should submit curriculum vitae, statement of research, extramural funding plans, and three letters of reference to:

Dr. Steffen Rogers ■ Director, The Mervin Bovaird Center
The University of Tulsa ■ 600 South College Avenue, Tulsa, Oklahoma 74104

The University of Tulsa has an Equal Opportunity/Affirmative Action Program for students and employees.

(NW4499)A

THE INSTITUTE OF CANCER RESEARCH

(University of London)

Sutton, Surrey

SCIENTIFIC OFFICER

MEDICINE

A Scientific Officer is required to work on a project to investigate the use of the polymerase chain reaction to detect micrometastatic disease in lymphocytic lymphoma and myeloma, which will be an integral part of the clinical programmes for these diseases.

The post should provide experience in a wide variety of molecular biology and tissue culture tecnhiques. The research is hoped to create the necessary interface between laboratory and clinical research.

Applicants must hold a degree or equivalent qualification and be able to work as a member of a small team. Experience in molecular biology an advantage but not essential.

Salary will be in the range of £10,447 p.a. to £13,724 p.a. (inclusive). The post is offered initially on a two year contract.

For further information please contact Dr. D. Cunningham tel: 01-643 8901 ext. 3281.

Please forward C.V., in duplicate, with the names and addresses of two referees to the **Personnel Officer, The Institute of Cancer Research, 17A Onslow Gardens, London SW7 3AL** quoting ref no. 10:89.T N 52

Applicants are advised that smoking is prohibited in the majority of the Institute's premises. (2003)A

ERATO

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RESEARCH ENGINEERS AND SCIENTISTS: You are invited to COME TO JAPAN to do research at the forefront of basic science and engineering. ERATO, a program fully funded and administered by the Research Development Corporation of Japan, a statutory corporation of the Japanese government, has been inviting non-Japanese researchers to Japan since 1981. ERATO is now seeking researchers for the projects below. (ERATO Projects are directed and funded independently from the project directors' respective parent affiliations).

TONOMURA ELECTRON WAVEFRONT (1989-1994) — Experimental and theoretical physicists, image processing engineers, and biologists will research the basic physics, instrumentation, and applications of electron holography.

Project Director: Akira Tonomura, Chief Researcher, Advanced Research Laboratory, Hitachi, Ltd.

AONO ATOMCRAFT (1989-1994) — Surface scientists, experimental and theoretical physicists and chemists, micromechanical and control engineers, and computer scientists will research the direct manipulation of atoms and molecules on solid surfaces.

Project Director: Masakazu Aono, Chief Scientist, RIKEN (The Institute of Physical and Chemical Research)

IKEDA GENOSPHERE (1989-1994) — Molecular biologists, human geneticists, molecular cytologists, and optoelectronics engineers with interest in the laser measurement of chromosomes will identify, dissect, and analyze human genes relating to cancer and other diseases.

Project Director: Joh-E Ikeda, Chief, Molecular Genetics Laboratory, National Institute of Agrobiological Resources

SAKAKI QUANTUM WAVE (1988-1993) — Solid state physicists, solid state chemists, and semiconductor engineers are researching the fabrication and behavior of quantum wells and wires, structures with features smaller than the electron's mean free path.

Project Director: Hiroyuki Sakaki, Professor, Research Center for Advanced Science and Technology, University of Tokyo

MASUHARA MICROPHOTOCONVERSION (1988-1993) — Electrochemists, physical chemists, applied physicists, and electronic engineers will research the use of lasers to drive, measure and control pico-second chemical reactions in fabricated micrometer-size volumes.

Project Director: Hiroshi Masuhara, Professor, Kyoto Institute of Technology

NISHIZAWA TERAHERTZ (1987-1992) — Solid state physicists, electron physicists, electronic engineers, solid state chemists, and communications engineers are researching the fabrication, behavior, and measurement of semiconductor devices for operation at terahertz frequencies.

Project Director: Jun'Ichi Nishizawa, Professor, Tohoku University, and Director, Semiconductor Research Institute, Sendal

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For an independent review of ERATO, see "JTECH (Japanese Technology Evaluation Program) Report on ERATO," National Science Foundation, Dec. 1988 — available from NTIS, 5285 Port Royal Road, Springfield, Virginia 22161 USA, Telephone (703) 487-4826.

Please send your resume and lists of publications and references to ERATO, c/o

ISTA

ERATO Overseas Representative

International Science & Technology Associates, Inc.
11Beatrice Drive, Bryn Mawr, Pennsylvania 19010-2301 USA
Telephone: (215)527-4538, Facsimile: (215)527-2041 (6671)A

MEDICAL RESEARCH COUNCIL
Laboratory of Molecular Biology
Cambridge

RESEARCH ASSISTANT

Applications are invited from research orientated graduates to work in a research group with Dr. Richard Henderson. The research concerns the structure of membrane receptors coupled to G-proteins involved in signal transduction (rhodopsin, ß-adrenergic receptors and related receptors). These molecules all contain seven transmembrane helices and their structure is important for understanding of the action of many existing drugs and for the development of more specific drugs in the future.

Practical work will include membrane receptor protein expression in eucaryotic and procaryotic systems, functional analysis of the products and development of large scale purifications. Candidates will benefit from practical experience in biochemistry and molecular biology.

For more information contact directly Dr Richard Henderson or Dr Gebhard Schertler (phone 0223 248011). Application, including a CV and the names of two professional referees, should be sent by Friday 27 November 1989 quoting reference SS 7196 to:

The Personal Officer,
MRC Laboratory of Molecular Biology,
Hills Road,
Cambridge,
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(1949)A

SCHOOL OF BIOLOGICAL SCIENCES

RE-ADVERTISEMENT

Postdoctoral Research Assistant/Postdoctoral Research Fellow in Molecular Biology

(SALARY R. & A. IB scale :– £9,816–£12,381)
or (R. & A. IA scale :– £10,458–£12,381)

Applications are invited for the above M.A.F.F.-funded post, available immediately and tenable for 3 years. The research will be carried out within the Microbiology and Molecular Biology Research Group in the School of Biological Sciences in collaboration with the School of Agriculture and Forest Sciences.

Candidates should ideally have a background in Recombinant DNA research. The successful applicant will be involved in a major programme (including a grant-funded research technician) using molecular biology techniques to identify the gene(s) determining fertility in sheep.

Salary for Postdoctoral Research Assistant on the R. & A. IB scale within the range :– £9,816–£12,381 or for the Postdoctoral Research Fellow on the R. & A. IA scale:– £10,458–£12,381 depending upon qualifications.

Applications (two copies) giving details of age, education and experience together with the names and addresses of two referees should be sent to **Mr. Alan James, Personnel Officer, University College of North Wales, Bangor, Gwynedd LL57 2DG**, by not later than 17th November 1989. Informal enquiries may be made to Dr. S.J. Assinder, tel. no. (0248) 351151, ext. 2604. (1980)A

Prifysgol Cymru · University of Wales
BANGOR

British Geological Survey

CHEMIST

The Survey has a vacancy for a Chemist in its Fluid Processes Research Group based at Keyworth, Nottingham

The Group is a multi-disciplinary and enthusiastic unit involved in a range of studies into the processes controlling the fate of pollutants in the environment BGS has well-appointed radiochemical and analytical laboratories and requires a chemist with an aptitude for innovative research into a range of environmental problems The successful candidate will initially assist in studying the transport and specification of radionuclides in groundwater and their interactions with mineral surfaces This is presently being approached by the study of natural systems supported by controlled field and laboratory radiotracer experiments

Candidates should have a good honours degree in chemistry preferably with research experience in one or more of the following areas - Electrochemistry, Radiochemistry, Organic Geochemistry (HPLC,GC), Colloid or Surface Chemistry Scientific development and training are encouraged

Salary will be in the range of **£9,131** to **£14,909** according to qualifications and experience Terms include a generous leave allowance and a non-contributory pension scheme The appointment is for a period of three years with the possibility of an extension

The British Geological Survey is a component body of the Natural Environment Research Council

For an application form please write to - Establishments (Recruitment), British Geological Survey, Nicker Hill, Keyworth, Nottingham NG12 5GG Please quote reference SO/FP/89/3 Closing date for completed application forms is 24th November 1989

The Natural Environment Research Council is an Equal Opportunities employer

Natural
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(1974)A

UNIVERSITY OF GUELPH

Department of Biomedical Sciences

RESEARCH ASSOCIATE

Cryobiology of Mammalian Embryos

An experienced cryobiologist is sought to lead the cryopreservation sector of a research and development program involving the manipulation of bovine embryos The appointment will be for a 4-year term initially

In accordance with Canadian immigration requirements, this advertisement is directed to Canadian citizens and permanent residents of Canada The University of Guelph is committed to employment equity

Applications, including a curriculum vitae and the names of two academic referees, should be sent to **Professor K J Betteridge, Department Biomedical Sciences, University of Guelph, Guelph, Ontario, N1G 2W1** (Tel 519 824-4120, ext 8258, FAX 519 767-1450) before December 31, 1989

(NW4495)A

FACULTY POSITION IN MOLECULAR IMMUNOLOGY
DEPARTMENT OF BIOCHEMISTRY AND BIOPHYSICS
IOWA STATE UNIVERSITY

The department invites applications for a tenure-track position at the level of assistant or associate professor The successful applicant will be expected to develop an independent research program in some molecular aspect of the immune system, possible areas of interest include, but are not limited to, protein chemistry, molecular structure, and molecular genetics The appointee will be expected to have an interest in interacting with existing research programs in the department, to assume moderate teaching duties, and to participate in the interdepartmental Immunobiology Program Generous start-up funds and laboratory space in the new Molecular Biology Building are available Applicants should send curriculum vitae, statement of research plans, and the names and addresses of three references by December 15, 1989 to

Dr Alan M Myers, Department of Biochemistry and Biophysics, 397 Gilman Hall, Iowa State University, Ames, IA 50011

Iowa State University is an Affirmative Action/Equal Opportunity Employer Applications from women and minority candidates are especially encouraged (NW4480)A

University of Freiburg i. Br. (FRG)

Institute of Mineralogy and Petrology

Applications are invited for a post-doctoral research and teaching assistant position (C1) in mineralogy/petrology The 3-years appointment can be extended to a maximum of 6 years The position is available immediately Candidates should have a Ph D degree and a record of significant contributions to the fields of Mineralogy, Petrology or Geochemistry A strong background in physical chemistry and/or computer sciences is desired

The successful candidate will participate in research projects related to fluid/rock interaction processes in the crust

Applications with curriculum vitae and a list of publications should be submitted no later than November 30, to **The Institute of Mineralogy and Petrology, University Freiburg i Br., Albertstr. 23b, D-7800 Freiburg i Br , FRG**

(W6660)A

UNIVERSITY OF SOUTHAMPTON

CRC Wessex Regional Medical Oncology Unit

GROWTH FACTOR PROJECT

Applications are invited for the position of Research Assistant in a group studying the role of polypeptide growth factors in the development and dissemination of cancer

Applicants should have a degree or equivalent in a biological science with a strong background in biochemistry or cell biology Some experience of tissue culture techniques would be an advantage

This is a Cancer Research Campaign funded appointment initially for 2 years, salary range (£9,816–£12,381) according to age and experience

Applications (2 copies) including a full C V and the names and addresses of 2 referees to **Ms J A Doyle, Staffing Department, University of Southampton, Southampton, SO9 5NH,** by 23 November 1989 *Please quote reference number 120/JAD/jm* (1972)A

RESEARCH SCIENTIST

(Environmental Physics)

RESEARCH SCIENTIST/
SENIOR RESEARCH SCIENTIST

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Division of Water Resources
CANBERRA AUSTRALIA

GENERAL The CSIRO Division of Water Resources has a staff of 220, including 100 scientists, and conducts research and development into the definition, use and management of Australia's water resources The Division has laboratories in Perth, Adelaide, Canberra and Griffith

As part of research into hydrologic processes at a regional scale, a research group in the Division is concerned with tracing the sources of sediment reaching rivers and water storages This current source tracing uses mineral particle magnetics and radionuclides in catchments up to 56,000 km^2 in area

THE JOB The appointee will join a project team undertaking a number of sourcing investigations in catchments across the country These activities are strongly linked with the needs of soil and water management agencies The appointee will conduct research into the use of natural and anthropogenic radionuclides as tracers of both sediment sources and transit times in large catchments The radionuclides will be used in conjunction with mineral particle magnetics for tracing Communication with government agencies is an important element of the appointee's activities The appointee will be based in Canberra

QUALIFICATIONS A PhD in physics, chemistry or other science is required The appointee must be completely familiar with the principles, operation and use of nuclear electronics, and gamma and alpha high resolution spectrometers He/she must be able to analyse samples for their contents of the natural radioactive decay series, and anthropogenic nuclides The appointee must be able to independently manage all aspects of a radionuclide laboratory, with emphasis on routine high precision low level gamma spectrometry The appointee will also be expected to manage a VG Aqua-Sira mass spectrometer and so familiarity with stable isotope mass spectrometry is desirable Familiarity with the techniques of mineral particle magnetic tracing would be an advantage

TENURE Appointment will be for an indefinite period with Australian Government Superannuation benefits

MORE INFORMATION A job description and selection criteria are available from Mr Geoff Black, CSIRO Division of Water Resources 61 62 46 5213 Questions arising from these may be discussed further with Dr R J Wasson 61 62 46 5665

APPLICATIONS These should be submitted by 17 November 1989, quoting reference number A3135 They should address the selection criteria and include relevant personal and professional particulars including details of formal qualifications and experience Applicants should nominate at least two professional referees, and address applications to

The Chief
CSIRO
Division of Water Resources
GPO Box 1666
CANBERRA ACT 2601
AUSTRALIA

CSIRO
AUSTRALIA

AB1-LR24

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(W6678)A

KING'S COLLEGE LONDON

BIOMEDICAL SCIENCES DIVISION

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for a position as Postdoctoral Research Fellow to work with Dr K C Pedley and Professor S L Howell in an active research group studying the mechanism and regulation of insulin secretion The project will investigate the role of calcium and protein kinase C in stimulus-secretion coupling in pancreatic B cells, using digital imaging microscopy Relevant experience in one of these areas would be an advantage but is not essential This MRC-funded post is available immediately for a period of three years at a starting salary of £14,031 per annum, including London Allowance Applications and enquiries should be sent to **Professor S L Howell, Head of Biomedical Sciences Division, King's College London, Campden Hill Road, Kensington, London W8 9AH,** before 1 December 1989

(1988)A

BIOMERIEUX/CNRS

Department of Retrovirology

Senior Retrovirologist or Post Doctoral Position in Molecular Biology/Virology

A joint laboratory between the French CNRS and bioMerieux, a leading Diagnostic company, offers opportunities for a virologist or a molecular biologist willing to work on regulatory genes of HIV

The laboratory, fully equipped for molecular biology, is located in a new High School for education and research Other laboratories at the same location include molecular and cellular biology teams

Candidates should have a PhD as well as significant postdoctoral research experience and potential for developing an independent research programme

Interested applicants should forward a brief description of research plans, curriculum vitae and names of references to

B MANDRAND
UNITE MIXTE BIOMERIEUX/CNRS
ECOLE NORMALE SUPERIEURE DE LYON
46, allee d'Italie
69007 LYON
FRANCE
Phone number (33) 7272 8359
Fax number (33) 7272 8080

(W6664)A

MEDICAL RESEARCH COUNCIL
NATIONAL INSTITUTE FOR MEDICAL RESEARCH
GENES AND CELLULAR CONTROLS GROUP

POSTDOCTORAL SCIENTIST

Applications are invited for a short-term (three-year) postdoctoral appointment to the scientific staff, tenable in the Laboratory of Eukaryotic Molecular Genetics The research, which will be under the supervision of Dr N B La Thangue, will involve studies on the mechanisms of transcriptinal control during early murine development The project will use a wide range of molecular biological and immunochemical techniques to study the structure and function of an embryonic transcription factor Experience in the relevant techniques would be an advantage but is not essential as full training will be given

The position is available immediately Informal enquiries may be made by telephoning Dr La Thangue on 01 959 3666 Ext 2094 The salary will be in the range £12,381 to £20,469 (starting salary dependent on experience) plus £1,650 per annum London Allowance MRC Pension Scheme option

Applications including a curriculum vitae, list of publications and the names and addresses of two professional referees should be sent to **Mr C R Russell, Administrative Manager, National Institute for Medical Research, The Ridgeway, Mill Hill, London, NW7 1AA,** not later than 24th November 1989, quoting reference number GEKG/0579

The MRC is an Equal Opportunities Employer

(1982)A

INSTITUTE OF THEORETICAL GEOPHYSICS
UNIVERSITY OF CAMBRIDGE

UNIVERSITY LECTURESHIPS IN THEORETICAL GEOPHYSICS

Applications invited in any area of Theoretical Geophysics, to work in the new Institute of Theoretical Geophysics established between the Departments of Applied Mathematics & Theoretical Physics and Earth Sciences, under the Directorship of Professor H E Huppert, FRS The Institute plans to attract exceptionally able theoretically inclined scientists whose research is in any area of the application of physics and mathematics to understanding the Earth, which includes at least the topics of geodynamics, geological fluid mechanics, geomagnetism, mantle mineral physics, meteorology, oceanography and seismology

Salary scale for £14,169–£21,850

Applications, including c v (10 copies), and names of three referees, by *31 December, 1989* to the **Administrator, Department of Earth Sciences, Downing Street, Cambridge CB2 3EQ, U K**

(1971)A

INIVERSITY OF DURHAM

PROFESSOR OF 'LANT SCIENCES

epartment of Biological Sciences

pplications are invited for the 'st of Professor of Plant Sciences the Department of Biological iences at Durham University

ofessor D Boulter will retire in ptember 1991 and his successor ll be appointed in the same field, as to build upon the established putation for science at Durham in ant Molecular Biology and Plant ochemistry

e starting date will be 1 February 91, or as soon thereafter as nvenient

rther particulars may be obtained m the **Registrar and Secretary), University of Durham, Old ire Hall, Durham DH1 3HP,** to om applications (twelve copies), cluding the names of three refees, should be submitted not ter than Thursday 30 November 89

andidates outside the British es need submit one copy only)

(1989)A

Institute of Oceanographic Sciences

EARTH SCIENTIST/ PHYSICAL SCIENTIST

Wormley **Salary to £14,909 pa**

The Institute is looking for a Sea-going research scientist to join a team studying mid-ocean ridge tectonics using Side-Scan Sonar and other high resolution surveying techniques Applicants should have a good honours degree and preferably an MSc/PhD Conditions of service for this permanent post include a generous annual leave entitlement and a non-contributory pension scheme Further details may be obtained from Mrs R Stacey, Institute of Oceanographic Sciences Deacon Laboratory, Brook Road, Wormley, Godalming, Surrey GU8 5UB Telephone 042 879 4141 Closing date for applications is 23rd November 1989 Quote reference number G18

The Natural Environment Research Council is an Equal Opportunities employer

Natural
Environment
Research
Council

(1975)A

Medizinische Universitatsklinik
(Freiburg University Medical Center)
Freiburg im Breisgau/West Germany

A POSTDOCTORAL POSITION IN MOLECULAR IMMUNOLOGY

is available in the beginning of 1990 for candidates with a strong background in molecular genetics Existing research in the laboratory included the analysis of the terminal maturation of human monocytes into macrophages, and studies on the regulation of monocytic cytokine and cytokine receptors expression (publications in Blood and J Exp Med). Scientists who are willing to work on the molecular cloning of receptors are encouraged to apply The appointment will be for three years Appropriate salary and start-up funds are available A detailed curriculum vitae, including a list of publications, and two letters of recommendation (or the names and telephone numbers of two referees) should be addressed to **Joachim Bauer, M D , Medizinische Universitatsklinik, Labor B 13; D-7800 Freiburg, West Germany.** *Closing date 30th November 1989*

(W6662)A

BIOLOGICAL OCEANOGRAPHER

The Department of Organismic and Evolutionary Biology and the Committee on Oceanography at Harvard University seek to make an appointment in biological oceanography at the rank of assistant professor Preference will be given to candidates with interests in phytoplankton processes In addition to research interests in oceanography, the applicants should be prepared to teach oceanography at the undergraduate and graduate levels, and in the area of phycology at the introductory level Applicants should send a curriculum vitae, statement of research interests, and the names of three references to **James J McCarthy, Chairman, Search Committee for Biological Oceanography, MCZ, Harvard University, Cambridge, MA 02138**

Harvard University is an Affirmative Action/Equal Opportunity Employer (NW4452)A

UNIVERSITY OF LEICESTER
Department of Pharmacology & Therapeutics

CLINICAL SENIOR LECTURESHIP IN PHARMACOLOGY

New Appointment Scheme for Clinical Academic Staff (NASCA)

Applications are invited for the post of Clinical Senior Lecturer in Pharmacology This post will be the third Senior Lectureship in the Department The expansion is intended to further integrate the activities of the clinical with the basic science groups of the Department, which has an international reputation in molecular aspects of receptor pharmacology

The appointee will be expected to pursue laboratory based research interests preferably in the fields of biochemical/molecular pharmacology or molecular biology and work in collaboration with the basic science groups and the clinical scientists in the Department whose special interest is cardiovascular medicine Integration of research activity is also possible with the Interdisciplinary Research Centre associated with the MRC Human Toxicology Unit which is being established in Leicester

It is anticipated that the Leicestershire Health Authority will grant an honorary consultant contract to a suitably qualified applicant who will be based at the Leicester Royal Infirmary

Initial salary will depend on qualifications and experience on the scale £29,700 to £38,340 p a

Further particulars from the Staffing Office (Academic Appointments), University of Leicester, University Road, Leicester LE1 7RH, (tel 0533 5222439), to whom applications on the form provided should be sent by 1 December 1989 Informal enquiries are welcome contact Professor D B Barnett, Department of Pharmacology, Leicester Royal Infirmary, LE2 7LX (tel 0533 523126) or Professor S R Nahorski, Department of Pharmacology, University of Leicester (tel 0533 522923) (1993)A

1990-91 visitor appointments in the High Altitude Observatory (HAO)

at the National Center for Atmospheric Research are available for up to one year in solar physics, solar-terrestrial physics, and related astrophysics We encourage scientists with strong backgrounds in physics (experimental and theoretical) to apply Due by 1/15/90 are 1) vita, 2) list of publications, 3) recommendation letters from three scientists familiar with applicant's work, 4) statement of proposed research at HAO, 5) statement of financial needs, number of family members accompanying you, ages of children, 6) transcripts of graduate courses and Ph D thesis abstract if less than two years' post-Ph D experience Send to **HAO Visitors Committee, HAO/NCAR, P O Box 3000, Boulder, CO 80307-3000,** NCAR is an EO/AA employer (NW4180)A

Faculty Position

Molecular Biology of Aging Skin

The University of Michigan Medical Center has a faculty position available at the research investigator level to study the molecular basis of aging and the effects of retinoids (vitamin A-like molecules) on aging of human skin The successful candidate will have a Ph D and/or M.D degree in cellular/molecular biological techniques, including DNA sequencing, nucleic acid hybridization, RNA isolation, molecular cloning, and transfection of eukaryotic cells

Fully equipped laboratory and support facilities are available Position includes funding for supplies and a research assistant.

The University of Michigan is an equal opportunity employer

John J. Voorhees, M.D.
Professor and Chairman
Department of Dermatology
Room 1910 Taubman Center
The University of Michigan Medical Center
1500 East Medical Center Drive
Ann Arbor, Michigan 48109-0314

(NW4493)A

PHYSICAL/SURFACE CHEMIST

The Brewing Research Foundation, a privately funded organisation with an international reputation as a centre of excellence, requires a Physical/Surface Chemist to work in its Beer Quality Section The successful candidate will research into various aspects of beer foam including the development of a new method of measuring foam quality

This post is particularly suitable for a chemist with a Ph D degree in chemistry although candidates with a good first degree in chemistry are also invited to apply Conditions of service are good and initial salaries depend upon experience and qualifications

Applications in writing, enclosing a curriculum vitae and the names of two referees to

The Administrator,
Brewing Research Foundation,
Lyttel Hall, Nutfield, Redhill, Surrey,
RH1 4HY

quoting reference BQ3 (1981)A

ASSISTANT PROFESSOR — IMMUNOLOGY

Department of Veterinary Microbiology & Immunology, School of Veterinary Medicine, University of California is seeking a highly qualified applicant to fill a tenure track position in Immunology Qualifications include the Ph D degree and advanced training in cellular and/or molecular biology with demonstrated research productivity and interest in Immunology The successful candidate will be expected to contribute to the Department's and the School's Immunology teaching commitments and to establish independent and collaborative-type research programs Apply in writing and include CV, list of publications, and names of three references Send to **Dr Jeffrey L Stott, Chairperson Search Committee, Department of Veterinary Microbiology & Immunology, University of California, Davis, CA 95616** Appications accepted through JANUARY 15, 1990 or until a suitable candidate is identified

THE UNIVERSITY OF CALIFORNIA IS AN AFFIRMATIVE ACTION/EQUAL OPPORTUNITY EMPLOYER (NW4476)A

MEDICAL RESEARCH COUNCIL:

NON CLINICAL SCIENTIST

The Neuro-otology section of the Human Movement and Balanc Unit has a vacancy for a non-clinical scientist who will be responsibl for research in human balance and spatial orientation Specificall candidates should have training or experience relevant to one o more of the following physiological, psychological, medical or bio engineering aspects of eye movements and optics, vestibular function, sensory-motor control of balance and movement, visuo-spatia perception, man-vehicle interaction

The successful candidate will be expected to participate in a researc programme directly relevant to problems of patients with diseases o the ears, eyes and nervous system affecting balance, orientation an mobility in collaboration with an interdisciplinary team of physician and scientists

Candidates are expected to have at least a good honours degree o equivalent in the physical or biological sciences or engineering Th salary will be on the scale for University Lecturers within the rang £10,458-£20,469 plus London weighting of £1,650 The appointmen will be made for 3 years For application form and job descriptio please write quoting reference number (7150) to **The Secretary, Section of Neuro-otology, MRC Human Movement and Balance Unit, National Hospital, Queen Square, London WC1N 3BG**

(1963)A

MRC
Medical Research Counci

UNIVERSITY OF NOTTINGHAM

SCHOOL OF AGRICULTURE
SUTTON BONINGTON, NR LOUGHBOROUGH
Department of Physiology and Environmental Science

TECHNICIAN GRADE 3

Applications are invited for the above position, available immediately and funded initially for a period of 12 months, in an activ research group concerned with the neurophysiology of the spina cord In addition to participating in the day to day maintenance o the laboratory, the successful applicant will be encouraged t develop histological/histochemical skills applied to the central nervous system and be prepared to learn allied neurophysiological techniques Some experience in histological processing and/or neurophysiology would be an advantage but is not essential Salary in accordance with Grade 3 scale for technicians, £7,479 – £9,171 per annum according to age, qualifications and experience, for a 5-day, 37 hour week

Application forms obtainable from **Mr P G Ramsey, Administrative Assistant, University of Nottingham, School of Agriculture, Sutton Bonington, Nr Loughborough, Leices, LE12 5RD** Telephone Nottingham 484848 Ext 8302 Please quote post ref 89/21 (1970)A

ASSOCIATE PROFESSOR

CELL PHYSIOLOGY

The Department of Physiology at the University of Minnesota invites applicants for a tenured appointment at the level of Associate Professor in Cell Physiology beginning immediately Selection will be based primarily on demonstrated excellence in an established, independent research program that is compatible with other departmental programs Preference will be given to the area of vision neurophysiology at the retinal level

A tenure appointment requires that the applicant demonstrate a national and international reputation in research and experience and proficiency in teaching The applicant will be expected to participate in the teaching and graduate education programs in the department The last date for receipt of applications has been extended to December 15 Send curriculum vitae and a description of current research program to **Dr Richard Poppele, Department of Physiology, 6-255 Millard Hall, 435 Delaware Street SE, University of Minnesota, Minneapolis, MN 55455**

The University of Minnesota is an equal opportunity educator and employer and specifically invites and encourages applications from women and minorities

(NW4479)A

Career Opportunities In Neuropharmacology

Ware, Herts

Does career progression in your current job feel like an obstacle course? If so, then look instead at the benefits of joining a team with a proven track record in a company determined to help you succeed

Glaxo Group Research is Britain's leading pharmaceutical company and as you would expect offers impressive benefits too Whether it's performance-related pay to reward your efforts, or promotion on merit to recognise your abilities, you'll find that we are determined to give you the chance to advance Working in a confident and vigorous research climate may well be what you're missing too

Which ever reason may apply to you, we have the solution - expansion into new areas means that we are seeking graduates for our Neuropharmacology department at Ware This is an excellent opportunity to join a highly productive research group and work with young scientists who lead in their field Some of them studied part-time for a PhD while working at Glaxo - and that opportunity will be extended to you too

If you have up to 3 years relevant experience, preferably in industry, following a degree in Pharmacology or an allied subject we would like to hear from you

Write or telephone for an application form, quoting reference W89221. The Research Personnel Officer, Glaxo Group Research, Park Road, Ware, Herts SG12 ODP. 0920 469469 Ext. 2348

Glaxo Group Research

(2007)A

MOLECULAR BIOPHYSICAL CHEMISTRY FACULTY POSITIONS

THE UNIVERSITY OF TEXAS MEDICAL BRANCH
GALVESTON, TEXAS

'he University of Texas Medical Branch at Galveston has :areer opportunities for three tenure-track Molecular Bio-'hysical Chemistry Faculty positions in the Human Biological :hemistry and Genetics Department UTMB employs 8,400)eople and consists of four schools, two research institutes ınd seven hospitals

[he University and local private foundations provide excel-ent support for equipment, research efforts, as well as newly 'enovated laboratory space Salaries are highly competitive ınd commensurate with qualifications and experience

3uccessful candidates will provide leadership and will inter-ıct with other faculty members, specially, a newly appointed ?obert A Welch Professor, in developing a center of scientific ›xcellence for applying molecular biophysical chemistry in ;olving biological problems Quality research will be the ›rimary criterion Research experience in any of biochemis-:ry and biophysics will be a definite plus

\pplications should include a complete curriculum vitae, a ;ummary of future research plane and names of at least three 'eferences Applications must be received by January 19, 1990 and should be addressed to

E Brad Thompson, M D
I H Kempner Professor and Chairman
Department of Human Biological Chemistry and Genetics
The University of Texas Medical Branch
Galveston, Texas 77550

UTMB is an equal opportunity M/F/V/H affirmative action ɘmployer UTMB hires only individuals authorized to work in the United States (NW4514)A

UNIVERSITY OF CAMBRIDGE

MOLECULAR BIOLOGIST

Applications are invited for a postdoctoral position funded by the Cancer Research Campaign to study the functions of the *fos* protein and its interactions with other transcription factors The post is available immediately and is funded for 5 years The work will be carried out at the Department of Pathology, Division of Virology until January 1991 when the group will relocate to the new Wellcome Trust/Cancer Research Campaign Institute of Cancer and Developmental Biology currently under construction in Cambridge Salary on age related University Research Associate scale

Further enquiries to Dr T Kouzarides Tel 0223 336922

Applications in writing with c v and names and addresses of two referees to **The Superintendent, Department of Pathology, Tennis Court Road, Cambridge CB2 1QP** Please quote Ref 11678

(1967)A

The Department of Neurology at the University of Michigan is seeking applications for a **Research or Tenure Track Position** in Molecular Neurobiology and/or protein chemistry Successful candidate will join a multi-disciplinary team of investigators studying neurotransmitter receptors Position includes laboratory space, start-up funds, and salary support for initial years of program Opportunity exists for a joint appointment in a basic science department Curriculum vitae and three letters of recommendation should be sent to **Robert L Macdonald, MD, PhD, Department of Neurology, University of Michigan, 1108 Neuroscience Bldg , Ann Arbor, MI** 48109-1687 *The University of Michigan is an equal opportunity/affirmative action employer and encourages women and minorities to apply* (NW4503)A

POSITIONS AVAILABLE

The International Laboratory for Research on Animal Diseases (ILRAD), is an internationally funded, major institution with the mandate of developing improved methods to control two important diseases of livestock, trypanosomiasis and theileriosis The multidisciplinary research program includes biochemistry, molecular biology, immunology, cell biology, pathology, parasitology, electron microscopy and entomology with appropriate supporting units Applications are invited for the positions of –

Scientist — Immunology (Ref Sci/89/2)
A position is available to join and supervise a group elucidating the role of T-cells in the induction of immunity to, and the pathogenesis of, *Theileria parva* infections in cattle Studies are carried out both *in vivo* and *in vitro* and include investigation of the role of the bovine Class I, II molecules and the involvement of different T-cell receptors in controlling the specificity of bovine immune responses Candidates will preferably have a first degree in veterinary science, a doctorate in immunology and over five years of post-doctoral experience in ruminant immunology at the cellular level

Scientist — Tick Research (Ref Sci/89/3)
A position to manage the Tick Vector Unit which provides an essential service to the *Theileria* research program The work entails maintenance and supervision of tick colonies, production of infected ticks, sporozoites and theilerial stabilates, supervision and screening of infected cattle and research activities related to immunization of cattle against East Coast fever Candidates must have had experience with ticks and tick-borne diseases Preference will be given to applicants with a first degree in veterinary medicine and a doctorate in a relevant area of parasitology

Research Associate — Immunochemistry (Ref RA/89/2)
A position in immunochemistry is available to support a multidisciplinary reseach group investigating cellular immune responses of cattle to the protozoan parasite, *Theileria parva* The successful candidate will assist in identification of parasite antigens responsible for stimulating T cell responses to parasitised lymphoblasts and to develop antibody reagents which can be used in experiments to isolate and transfect parasite genes The person recruited will also be resonsible for supervision and training of junior technical staff

Applicants should have a science degree or an equivalent diploma in immunological or medical laboratory technology Extensive experience of techniques in protein biochemistry and analysis of antigen-antibody reactions is essential

Research Associate — Immunodiagnostics (Ref RA/89/3)
A research associate position is available in a group involved in the development of improved immunological techniques for the diagnosis of African trypanosomiasis Applicants will have advanced technical qualifications, or a degree in Biochemistry/Immunology with at least 5 years' experience in a senior technical position with supervisory and training responsibilities Consideration will be given only to those applicants with proven abilities in the development and application of immunodiagnostic techniques, and practical experience with antigen and antibody purification procedures, enzyme and other labels, and monoclonal antibody production

These are International staff positions and salary will be paid in US dollars Initial salary will be determined by qualifications and experience Scientific staff are expected to contribute to the training program Moving, housing, commutation and education allowances are paid and there are medical and retirement benefits Applicants should include current curriculum vitae and names and addresses of three referees Applications, citing the position reference number, should be sent by December, 15th 1989 to

The Chief Personnel Officer,
I L R A D,
P O BOX 30709,
NAIROBI
Kenya

(W6673)A

UNIVERSITY OF WYOMING
ASSISTANT PROFESSOR
MOLECULAR BIOLOGY

The Department of Molecular Biology at the University of Wyoming has a tenure-track assistant professor position available July 1, 1990 Candidates should have at least two years of postdoctoral experience, will be expected to establish and maintain an active research program, and be involved in teaching at both the undergraduate and graduate levels Applicants in all areas of molecular biology/biochemistry will be considered although preference will be given to candidates with a biophysical orientation The Department has 15 faculty with active research programs funded by NIH, NSF, DOD, USDA and EPA Salary is competitive

Applications will be reviewed starting January 1, 1990 and continued until position is filled

Applicants should send their curriculum vitae, summary of research plans, and arrange to have three letters of reference sent to **Ivan I Kaiser, Search Committee Chair, Department of Molecular Biology, Box 3944, University Station, Laramie, WY 82071–3944**

An Affirmative Action/Equal Opportunity Institution (NW4513)A

UNIVERSITY OF THE WEST INDIES
TRINIDAD
BREEDER/GENETICIST

Applications are invited for the post of Breeder/Geneticist in the UWI, Cocoa Research Unit Applicants should have a background in quantitative and population genetics with a sound knowledge of statistics and the use of microcomputers

The appointee will be expected to develop a research programme to study genetic relationships within the international cocoa germplasm collection and to relate these to plant breeding

Salary Scale Lecturer/Research Fellow TT$54,708 to 66,156

Unfurnished accommodation if available at 10% or furnished at 12½% or housing allowance at 20% of pensionable salary

Up to five full economy passages on appointment Study and travel grant Book allowance

Detailed applications naming three referees to registrar, **UWI, St Augustine, Trinidad** Closing date for application 1st December 1989 Details of post sent to all applicants (W6675)A

New York University Medical Center
POSTDOCTORAL FELLOW
Department of Pathology

Funding is available for a postdoctoral position tenable for two years The project involves the study of events that take place during processing and presentation of antigens by antigen presenting cells Emphasis will be placed on tracing the intracellular transport of antigens and characterizing the intracellular compartments involved in processing Suitable candidates will have a background in either cell biology or immunology

Interested applicants should submit a curriculum vitae including the names and addresses of two references to **Dr Stephanie Diment, Department of Pathology, New York University Medical Center, 550 First Avenue, New York, NY 10016** (NW4505)A

THE DAVID MAHONEY INSTITUTE OF NEUROLOGICAL SCIENCES (INS)

of the University of Pennsylvania invites applications for a tenure track position in systems neurobiology at the rank of **Assistant Professor** Ideally, the candidate should be working at the tissue level, perhaps with a "slice" preparation, and should be able to interact with and strengthen our current substantial representation in research on vision and learning and on neural development Candidates should (by 12/15/89) send a curriculum vitae, a statement of current research interests, and the names of three individuals willing to provide letters of evaluation to **Professor Peter Sterling, Systems Neuroscience Search, Department of Anatomy, University of Pennsylvania, Philadelphia, PA 19104-6058** EEO/AA Employer

(NW4510)A

RADIOCHEMIST/NUCLEAR CHEMIST
National Institutes of Health
Public Health Service

National Cancer Institute
Division of Cancer Treatment
Clinical Oncology Program
Radiation Oncology Branch

Qualifications Position involves research in labelling monoclonal antibodies with metallic radionuclides for clinical use in tumor diagnosis and therapy A qualified applicant should have

- A Ph D in Nuclear Chemistry or Radiochemistry
- Experience in working with high levels of radioactivity
- Ability to do careful scientific measurements
- Knowledge of radiochemical separations/and radionuclide purification

Biological or Radiopharmaceutical experience is desirable

Salary Dependent upon experience and qualifications

Location of Position Bldg 10, National Institutes of Health, Bethesda, Maryland

For further information contact

Dr Otto A Gansow
National Institutes of Health
Building 10, Room B3B69
9000 Rockville Pike
Bethesda, Maryland 20892
Telephone (301) 496-5936

U S Citizenship Is Not Required

NIH IS AN EQUAL OPPORTUNITY EMPLOYER
(NW4320)A

BASIC BIOMEDICAL FACULTY POSITIONS

Three faculty positions at the Assistant or Associate Professor level are available effective Feb 1, 1990 in several departments associated with the newly organized Division of Cellular and Molecular Medicine at the University of California School of Medicine at San Diego

Areas of research interest include — but are not restricted to 1) processing, packaging and transport of primary gene transcripts, 2) control of protein and membrane traffic in eukaryotic cells, and 3) eukaryotic cell response to tissue growth factors Other promising fields of research will also be considered

Each applicant is expected to establish an active individual research program but interactions and collaborations with other faculty members of the Division or School will be welcome Each applicant is also expected to participate actively in teaching and research training of medical and graduate students

For candidates for an Assistant Professor position, an M D, Ph D or M D-Ph D degree, at least two years of postdoctoral training, and a strong record in well-organized research are expected Candidates for an Associate Professor position are expected to have already achieved an outstanding record in their field of research Rank and salary determined by qualifications and UC pay scales

Applications supported by a curriculum vitae, a list of publications, a summary of research planned for the immediate future and a list of 5 to 6 potential referees should be sent by February 1, 1990 to

George E Palade, M D
Dean Designate for Scientific Affairs
UCSD School of Medicine (M-002)
La Jolla, CA 92093-0602

UCSD is an equal opportunity-Affirmative Action employer (NW4527)A

THE NATIONAL RESEARCH COUNCIL
ANNOUNCES

POSTDOCTORAL & SENIOR RESEARCH ASSOCIATESHIP AWARDS

The awards sponsored by the National Research Council are available at the U S Army Institute of Dental Research The research opportunities include physiology and biochemistry of bone regeneration (which also includes studies of growth factors using bone cell cultures) purification and characterization of bone-inductive proteins and monoclonal antibodies, engineering of predictably degrading bone regenerating systems biodegradable polymer chemistry and microencapsulation and time-release drug delivery systems Doctoral degree in life sciences or biomedical sciences is required Annual stipend for recent graduates is $28,000 and is appropriately higher for those with more than 5 years of experience Relocation reimbursement and funds for professional travel are available, and a health insurance program is offered Awards are for 1 year with possible renewals to a total of 3

For further information contact
Dr Jeffrey Hollinger at (202) 576-3764 or
Dr Anshumali Chaudhari at (202) 576-2098
Their mailing address is
U S Army Institute of Dental Research
Walter Reed Army Medical Center
Washington, D C 20307-5300
For application materials, write to
Associateship Programs
(GR 430/NDR),
NATIONAL RESEARCH COUNCIL
2101 Constitution Avenue, NW
Washington D C 20418
FAX (202) 334-2759

Application Deadlines
are January 15 and August 15, 1990

(NW4521)A

TENURE-TRACK FACULTY POSITION
IN THE CENTER FOR CANCER RESEARCH AND DEPARTMENT OF BIOLOGY
MASSACHUSETTS INSTITUTE OF TECHNOLOGY

The Department of Biology and the Center for Cancer Research at MIT seek applicants for a tenure-track position at the level of Assistant or Associate Professor Candidates should be able to develop a creative research program in the Department's educational activities Applicants should send curriculum vitae, bibliography, a brief description of research interests, and the names of at least three references to

Dr Phillip A Sharp, Professor and Director
Search Committee Chairperson
Center for Cancer Research, Room E17-529
Massachusetts Institute of Technology
Cambridge, MA 02139

MIT is an Equal Opportunity/Affirmative Action Employer Qualified women and members of minority groups are encouraged to apply
(NW4507)A

POPULATION GENETICS

The Department of Systematics and Ecology at The University of Kansas invites applications for a tenure-track position in population genetics at the assistant professor level Applicants must have a strong theoretical background in population genetics, and a broad research interest in the development of theory and the application of theory to studies of natural populations Preference will be given to individuals with post-doctoral experience and outside funding Successful applicants will be expected to develop a vigorous research program, to teach graduate and undergraduate courses in population genetics and evolutionary biology, and to participate actively in the interdepartmental Genetics Program The salary range is $32,000–34,000 Please send curriculum vitae, copies of selected publications, at least three letters of reference, a statement of research interest and goals, and a description of teaching experience and interests by January 17, 1990, to **Dr Robert D Holt, Chair, Population Genetics Search Committee, Department of Systematics and Ecology, Haworth Hall, University of Kansas, Lawrence, KS 66045–2106** *An Equal Opportunity/Affirmative Action Employer* (NW4501)A

TENURE-TRACK FACULTY POSITION
IN THE CENTER FOR CANCER RESEARCH AND DEPARTMENT OF BIOLOGY
MASSACHUSETTS INSTITUTE OF TECHNOLOGY

Candidates are sought for a tenure track position at the level of Assistant Professor in the field of biochemistry and molecular biology

Candidates should have significant research accomplishments in the area of contemporary cell biology We are particularly interested in young scientists with creative approaches to the biochemistry of proteins involved in cell regulation and genes encoding these proteins

The successful applicant will be expected to conduct an active research program in the Center for Cancer Research and to participate in the teaching program of the Department

Applicants should send curriculum vitae, a summary of their proposed research program, and the names of at least three scientists who can provide an evaluation of the candidate's past accomplishments and future potential

Send applications to

Dr Phillips W Robbins, Professor
Search Committee Chairperson
Center for Cancer Research, Room E17–233
Massachusetts Institute of Technology
Cambridge, MA 02139

— An Equal Opportunity/Affirmative Action Employer —
(NW4529)A

UNIVERSITY OF LEICESTER
POSTDOCTORAL RESEARCH ASSOCIATE IN ORGANIC CHEMISTRY

Applications are invited for a post-doctoral position, supported by Pharmachemie B V of Holland, to start as soon as possible The work will involve studies on the chiral synthesis of taxane natural products Applicants should have experience in and enthusiasm for the field of organic synthesis The appointment will be for one year on the RA IA salary range £10,458–£16,665 with a starting point depending on age and qualifications

Applicants should send a c v to **Dr P R Jenkins, Department of Chemistry, University of Leicester, Leicester LE1 7RH,** (tel 0533 522124) as soon as possible and they should ask two referees to write directly to him on their behalf
(2011)A

Senior Scientists in Biomedical Science — Kanagawa, Japan

We invite applications and/or nominations for the positions of senior scientists of the Institute for Biomedical Science The Institute, now being reorganized, will consist of four divisions and we call candidates for the following three divisions

1. Division of Molecular and Cell Biology
2. Division of Bioengineering and Biomedical Materials
3. Division of Artificial Organs and Organ Transplantation

The Institute is one of the three Centers constituting the parent center, Terumo R & D (Director General Tetsuzo Akutsu, M D , Ph D and 500 researchers) The new R&D building (44,146 sq meter) is under construction and will be open in December, 1989 It is located in the town of Nakai, Kanagawa Prefecture, one-hour drive away from Tokyo The town is situated on one of the best geographical environments to live in Japan with an excellent view of Mt Fuji in the west and Sagami Bay in the south

TERUMO CORPORATION is a leading manufacturer with an extensive product lineup including artificial organs, pharmaceuticals, transfusion and infusion products, medical electronics, labo products and medical disposables Terumo has manufacturing R&D and distribution facilities in Japan, U S A , Belgium and Australia

QUALIFICATIONS include a Ph D , and at least seven-year experience in one of the three above-described specialized areas, and an excellent record of scientific achievement Successful candidates will have a chance to be promoted to the Division Chief, who is expected to have supervisory responsibilities directing junior staff

SALARY will be commensurate with experience and qualifications We offer house accommodation, and benefits package together with the opportunity to work in our newly constructed superb facilities equipped with modern technologies

A letter of application, C V , list of publications, three professional referees, and your portrait should be sent by the end of November, 1989 to the following address

Yutaka Tomita, Managing Director,
Technical R & D Division,
Terumo Corporation,
44-1, 2-chome, Hatagaya,
Shibuya-ku, Tokyo 151, Japan
Phone 03(374)8020, Fax 03(374)7493

Applications will be reviewed continuously until suitable candidates are hired Promising candidates will be contacted for further discussions on the terms of employment contract

(W6595)A

TERUMO CORPORATION

THE FACULTY OF CHEMISTRY AND PHARMACY
Institute of Biochemistry
UNIVERSITY OF MUNICH

Applications are invited for the tenured position of an

ASSOCIATE PROFESSOR (C3) OF BIOCHEMISTRY

The applicant has to present the field both in teaching (lectures, courses) and research He/she should preferentially be working in the area of biochemistry of development or the structure and function of proteins There is an age limit of 52 years (at the date of appointment)

The University of Munich complies with an equal opportunities policy

Applications with the necessary academic qualifications (Ph D , teaching experiences, research record) are to be submitted with a **curriculum vitae, academic records** and **list of publications** to the **Dekan der Fakultat fur Chemie und Pharmazie, Universitat Munchen, Geschwister-Scholl-Platz 1, 8000 Munchen 22, F R G by January 31, 1990** (W6663)A

University of Pittsburgh

DEAN, GRADUATE SCHOOL OF PUBLIC HEALTH

The University of Pittsburgh seeks applications and nominations for the position of Dean, Graduate School of Public Health The School is one of six schools of the health sciences on the campus of the University and its affiliated hospitals The School enrolls 607 full- and part-time students (375 FTEs), studying for master's and doctoral degrees in the fields of biostatistics, human genetics, epidemiology, community health services, health administration, infectious diseases and microbiology, environmental health, occupational health, and radiation health The Dean is responsible to the Senior Vice President for the Health Sciences The annual expenditures of the School, including sponsored research, are in excess of $22,000,000

Candidates must have had substantial experience in the academic aspects of at least one of the fields of study at a level equivalent to a full professorship and preferably be engaged currently in research pertinent to that field

Nominations or resumes should be
sent before December 31, 1989, to

George M Bernier, Jr , MD
Chairman, Search Committee for
Dean, Graduate School of Public Health
M-246 Scaife Hall
University of Pittsburgh
Pittsburgh, PA 15261

An equal opportunity, affirmative action employer

(NW4491)A

ST. PATRICK'S COLLEGE, MAYNOOTH
DEPARTMENT OF BIOLOGY
(National University of Ireland)

POSTDOCTORAL RESEARCH

Applications are invited for a research position to be funded by the EC ECLAIR Scheme and which is expected to commence on December 1st 1989 The appointee will join a multidisciplinary group at Maynooth which is attempting to isolate environmentally adapted variants of entomopathogenic nematodes and their associated bacteria for use in the biological control of insect pests and the appointee will concentrate on nematode research Candidates should have experience in either nematology, cell biology, micromanipulation or genetics The partners associated with the research project are The Agricultural Genetics Co , Cambridge, U K and the Agarische Hogeschool, Friesland, Netherlands

The appointment will be for three years, salary scale IR£17,000-£18,700

Applications in the form of a CV and the names, addresses and telephone numbers of two referees should be sent to **Dr Ann Burnell, Biology Department, St Patrick's College, Maynooth, Co Kildare, Ireland**

(1986)A

KING'S COLLEGE SCHOOL OF MEDICINE AND DENTISTRY
of King's College London
POSTDOCTORAL RESEARCH FELLOW

Applications are invited for a three year appointment in the Department of Oral Pathology, funded by the Wellcome Trust

The project concerns the synthesis and processing of secretory proteinases in glandular cells Candidates should have a good background in biological sciences and experience of protein separation/hyridization techniques would be an advantage

Salary scale £12,108-£13,386 per annum inclusive of London Weighting Allowance

Further details my be obtained from the Secretary, Department of Oral Pathology, The Rayne Institute, 123 Coldharbour Lane, London SE5 9NU or by telephoning 01-274 6222 extn 4244/4245 Applications including a curriculum vitae and the names and addresses of two referees should be sent to the **Secretary of the School, Kings College School of Medicine and Dentistry, Bessemer Road, London SE5 9PJ,** by November 25th 1989

(1976)A

Do You Qualify for International?

CHEMISTS, LIFE SCIENTISTS, CHEMICAL ENGINEERS

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...¡El Mundo es Tuyo!
...Le Monde est à Vous!
...Die Welt ist Dein!
...世界はあなたのもの!

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Procter & Gamble has several entry level product and process development openings for BS MS or PhD Chemists Life Scientists and Chemical Engineers in Asia Europe Mexico and South America

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Procter & Gamble total sales are over 20 billion dollars world wide Major product categories include beauty care beverage detergent fabric care food health care household care paper and pharmaceutical consumer products Our technically based corporation spent over 600 million dollars in research and product development last year

We offer a stimulating environment for personal and professional growth highly competitive salaries and excellent benefits package including pension health care and paid relocation

If interested send your resume including country qualifications and language fluencies to

F O Schulz, Jr
International Openings
The Procter & Gamble Company
Ivorydale Technical Center (#1N)
Spring Grove Ave and June St
Cincinnati, OH 45217

PROCTER & GAMBLE

An Equal Opportunity Employer

(NW4488)A

UNIVERSITY OF SOUTHAMPTON SCHOOL OF MEDICINE

CRC RESEARCH GROUP IN GENETIC EPIDEMIOLOGY

Applications are invited for a postdoctoral fellow and a graduate research assistant to join an international collaborative programme in cancer genetics and linkage mapping of the human genome The work will involve collection and computer analysis of genetic and epidemiological data, using programs for segregation, association, and linkage analysis Opportunities will present themselves for interested persons to develop new methods of analysis Salary will be at the appropriate level on the Research 1A (postdoctoral) £10,458-£16,665 or 1B (graduate) £9,816-£12,381 scale Funding is initially available for up to 3 years Applications in writing, together with c v and names and addresses of two referees, should be sent to Professor N E Morton, to whom informal enquiries may also be made **Please quote reference number 119/JAD/jm** Closing date for applications is 30 November 1989

Department of Community Medicine, Southampton General Hospital, Southampton, SO9 4XY Tel (0703) 777222 extension 3768 (1990)A

DEPARTMENT OF BIOLOGY CASE WESTERN RESERVE UNIVERSITY

A tenure track faculty position at the assistant professor level is available in the area of

CELL, MOLECULAR BIOLOGY

Applicants will be considered with research interests in plant or animal systems, who have a strong commitment to research and undergraduate and graduate education

Interested persons should submit curriculum vitae plus a statement of current research interests, and have three letters of recommendation sent to **Dr Norman R Rushforth, Chairman, Department of Biology, Case Western Reserve University, Cleveland, OH 44106**

Case Western Reserve University is an Equal Opportunity/Affirmative Action Employer

(NW4485)A

Faculty Position

Molecular Biology of Aging Skin

The University of Michigan Medical Center has a faculty position available at the research investigator level to study the molecular basis on aging of human skin The successful candidate will have a Ph D and/or M D degree in cellular/molecular biological techniques, including DNA sequencing, nucleic acid hybridization, RNA isolation, molecular cloning, and transfection of eukaryotic cells Fully equipped laboratory and support facilities are available Position includes funding for supplies and a research assistant

The University of Michigan is an equal opportunity employer

John J. Voorhees, M.D
Professor and Chairman
Department of Dermatology
Room 1910 Taubman Center
The University of Michigan Medical Center
1500 East Medical Center Drive
Ann Arbor, Michigan 48109–0314

(NW4504)A

SIGFRIED AND JANET WEIS CENTER FOR RESEARCH GEISINGER CLINIC, DANVILLE, PA 17822

A Staff Scientist (equivalent to Assistant Professor/Associate Professor) or Senior Scientist (equivalent to Professor) position is available in the Weis Center for Research The Geisinger Medical Center is affiliated with Jefferson Medical College The Weis Center is located in a new research building (65,000 sq ft) that accomodates 20 separate laboratories The research building was completed in May, 1987 The focus of the Center is cellular and molecular aspects of cardiovascular function and disease Persons with expertise in molecular and cell biology including cloning, sequencing, and expression of genes, membrane proteins and receptors, and immunology and with an interest in research problems broadly relevant to the cardiovascular system will be given preference Scientists are provided institutional funds for their salary and support of postdoctoral fellows, research technicians, supplies and equipment Employment should begin on or after July 1, 1990 Applications will be accepted until the position is filled Applications should be addressed to **Dr Howard E Morgan, Senior Vice President for Research, Weis Center for Research, Geisinger Clinic, Danville, PA 17822-2601** A summary of current research, curriculum vitae, and list of professional referees should be included in the application EOE M/F/H/V

(NW4489)A

Progenics

Pharmaceuticals, Inc

INVESTIGATOR AND POSTDOCTORAL POSITIONS

The Departments of Molecular Immunology and Cancer Biology are recruiting scientists for investigator and postdoctoral positions Applications are invited from scientists of the highest caliber with Ph D , M D , or M D /Ph D degrees Progenics provides a strong interactive environment for collaborative research among basic science and clinical investigators Current research interests of the staff include

Paul J. Maddon, M.D., Ph.D.

Molecular biology and biochemistry of HIV and CD4 (the HIV receptor), molecular mechanisms of virus-receptor interaction and viral infection, development of viral inhibitors, molecular mechanisms of T cell function (Cell **42**, 93-104, **47**, 333-348, **54**, 865-874, **57**, 469-481, Nature **328**, 626-629, PNAS **84**, 9155-9159)

Gerard M. Housey, Ph.D.

Structure/function analyses of the isozymes of protein kinase C, development of domain-specific inhibitors, molecular mechanisms of oncogene action (Cell **52**, 343-354, PNAS **84**, 1065-1069)

Candidates should send a curriculum vitae, a description of their research experience, and the names and addresses of three references to

Personnel Director
Progenics Pharmaceuticals, Inc
Old Saw Mill River Road
Tarrytown
NY 10591

(NW4484)A

ASSISTANT CURATOR IN HERPETOLOGY

AMERICAN MUSEUM OF NATURAL HISTORY

The Department of Herpetology and Ichthyology invites applications for a tenure-track curatorial position The prime requisite is vigorous collection-based research in systematics of Recent amphibians and reptiles — with preference for a field-oriented specialist on tropical amphibians, lizards or snakes Must have a commitment to collection growth and curation, and be willing to assume administrative, education and exhibition responsibilities as appropriate

Salary will be commensurate with qualifications and experience The successful applicant must have completed all requirements for the Ph D by time of appointment Applicants should submit by 15 January, 1990 a letter of application with a brief statement of research objectives, curriculum vitae, complete bibliography, and names, addresses and telephone numbers of at least five references Submit to

Dr Charles W Myers, Chairman
Herpetology Search Committee
American Museum of Natural History
Central Park West at 79th Street
New York, N Y 10024-5192

An Equal Opportunity Employer

(NW4492)A

Faculty Positions Memorial Sloan-Kettering Cancer Center Cell Biology and Genetics Program

The Memorial Sloan-Kettering Cancer Cent
invites applications for several tenure-trac
junior and senior faculty positions in the Ce
Biology and Genetics Program This researc
program is one of four in the Institute, and
expanding in the areas of cell cycle control, di
ferentiation, cell structure, intercellular recogn
tion, and morphogenesis The Cell Biology an
Genetics Program is located in a new researc
building which also houses advanced faciliti
for protein and nucleic acid microchemistr
transgenic mice, and automated mammalian ce
micromanipulation The members of the Ce
Biology and Genetics programme faculty are af
liated with the Cell Biology Graduate Program
Cornell University Medical College The pos
tions offered are highly competitive with regar
to start-up funds, laboratory space, salary an
housing

Eligible candidates should have a doctor
degree, postdoctoral experience and demor
strate potential and ability to develop an inde
pendent, imaginative research program Form
applications are invited from junior as well a
established investigators Applications shoul
include a curriculum vitae, a list of publications,
summary of research activities and researc
plan, three letters of recommendation Applica
tions should be addressed to **Dr Joan Mas**
sague, c/o Christine Brohme, Box #135, Memor
ial Sloan-Kettering Cancer Center, 1275 Yor
Avenue, New York, N Y 10021

Equal employment opportunity/
Affirmative Action

(NW4481)A

FACULTY POSITION IN CELL BIOLOGY

The Department of Anatomy & Cell Biology at Columbia University, College of Physicians & Surgeons invites applications for a tenure-track position at the assistant professor level The applicant should have considerable promise and demonstrated achievement in any area of cell biological research, however, areas of particular interest include mechanisms of growth factor action, differentiation of vertebrate cells, and functions of the extracellular matrix The successful applicant will be expected to establish an outstanding, extramurally-funded research program and to contribute to the cell biological education of medical and graduate students

Candidates for this position should send curriculum vitae and a description of their proposed research program, and should arrange to have three letters of recommendation sent to **Faculty Search Committee, Department of Anatomy & Cell Biology, Columbia University, College of Physicians & Surgeons, 630 W 168th St , New York, NY 10032**

Columbia University is an affirmative action employer (NW4524)A

ST. MARY'S HOSPITAL MEDICAL SCHOOL

(a constituent College of Imperial College of Science, Technology and Medicine)
(University of London)
Norfolk Place, LONDON W2 1PG

LECTURER (NON-CLINICAL) IN CLINICAL ENDOCRINOLOGY

Applications are invited for this post, the person appointed will have major research responsibilities, and some teaching and administrative tasks The research interests of the department are currently in metabolism in diabetes, the control of pituitary hormone biosynthesis and release, and in biosensors applied to medicine Prospective applicants should contact Professor D G Johnston in the first instance (Tel (01) 725–1253)

Applications (6 copies) including full C V and names and addresses of 2 referees should be sent to **Personnel Department**, from where further written details should first be obtained, at the above address by 30 November

(1961)A

CELL BIOLOGY/NEUROBIOLOGY

A tenure-track position, at the assistant professor level, is available in the Biology Department at Wesleyan University for a person who is incorporating modern structural and molecular techniques into research addressing fundamental questions in neurobiology at the cellular level The position is made possible through a grant from the Howard Hughes Medical Institute Applicants should have postdoctoral experience and a demonstrated ability to develop an active research program Other responsibilities include directing the research of Ph D students and participating in education at the graduate and undergraduate level

Please send curriculum vitae, a statement describing a future research program, and arrange that three letters of recommendation be sent by 15 December 1989 to **Chair, Search Committee for Cell Biology/Neurobiology, Department of Biology, Wesleyan University, Middletown, CT 06457**

Wesleyan is an Equal Opportunity/Affirmative Action Institution We encourage applications from minority candidates and women

(NW4506)A

RICE UNIVERSITY

Department of Biochemistry and Cell Biology

FACULTY POSITIONS

Four tenure-track faculty positions (all levels) in cell, developmental and molecular biology and cellular neurobiology beginning between 1/91 and 9/91 Clear evidence of excellence in research is essential Review begins 1/90, and positions will remain open until filled Send CV, past/future research plans, and 3 letters of reference to **K S Matthews, Chair, Dept of Biochem and Cell Biology, Rice Univ , Houston, TX 77251**

Rice University is an Equal Opportunity/Affirmative Action Employer, women and minority candidates are encouraged to apply

(NW4474)A

Behavioural Pharmacologist and a postdoctoral research associate in behavioural pharmacology

Akzo, a leading multinational company with headquarters in Arnhem The Netherlands, currently employes over 70 000 people within 350 companies operating in 50 countries A position at the Akzo company means a career at a technologically high developed company with core activities in chemical products, man-made fibers, coatings and health-care products

Organon International B V., one of the 6 companies within the Pharmaceutical division of Akzo, develops and markets medicines for human use The total number of world-wide employees is about 6300, of which more than 1000 are involved in research and development The latter group conducts activities for the programmes Reproductive Medicine, Immunology & Oncology, Cardiovasculars and Central Nervous Systems Within our R & D Laboratories about 120 organic chemists, in several departments, are responsible for the design and synthesis of new drugs in many classes of compounds including peptides, steroids, heterocycles and carbohydrates

In our department for CNS-Pharmacology we need reinforcement of our team with a Behavioural Pharmacologist and a postdoctoral research associate in behavioural pharmacology Our research is aiming for new therapeutic agents for psychiatric and neurological diseases Major research topics in this department are antidepressants, antipsychotics, anti-dementia drugs, nerve growth enhancers, steroids and peptides Modern methods in neurobiology, neurochemistry, electrophysiology and animal behavioural sciences are exploited and kept up to date

We want our research scientists to join the front runners in their area of expertise, as new neurobiological approaches will have to be explored in order to come up with original and effective agents

We need persons with scientific ambitions and will provide them the facilities to enable original contributions to their area of research and to drug development Contract duration for the postdoctoral position which requires work on steroids and behaviour will be of limited duration

Informal inquiries may be made to Dr C L E Broekkamp by telephoning 31-4120-62801/61862

Letters of application accompanied by a full curriculum vitae and a list of publications should be submitted to Mrs W Cove, Personell Officer, Organon International B V, P O Box 20, 5340 BH Oss, The Netherlands

AKZO

(W6680)A

AMGUEDDFA GENEDLAETHOL CYMRU – NATIONAL MUSEUM OF WALES

BOTANY DEPARTMENT

QUATERNARY PALYNOLOGIST

REF RAB/QP

Applications are invited for this new post to further the work of the department on studying the evolution of the Welsh flora Interests in the ecology of upland habitats and in taxonomy would be an advantage

SALARY Research Assistant scale, currently £8,459 – £12,051 per annum – superannuated

Further particulars quoting the appropriate reference may be obtained from

The Personnel Officer,
National Museum of Wales,
Cathays Park, Cardiff, CF1 3NP,

(or by telephone on 0222/397951) to whom applications should be returned no later than 30 November 1989

(1985)A

Colaiste na hOllscoile Corcaigh
University College Cork

MICROBIOLOGY

Applications are invited for a full-time temporary post in Microbiology The appointment is for one year commencing on 1 January 1990

Preference will be given to a graduate with a PhD degree in Microbiology The duties involve lecturing, project supervision and research

Applicants are requested to forward a curriculum vitae and arrange that two referee's reports be forwarded to **Professor Seamus Condon, Food Microbiology Department, University College, Cork, Ireland** Tel Cork (010-353-21) 276871, ext 2396 *Closing date Friday, 17 November 1989*

(1984)A

THE SWISS INSTITUTE OF ALLERGY AND ASTHMA RESEARCH

is seeking a

POSTDOCTORAL RESEARCH FELLOW

with experience in immunology and cell biology preferably in the field of IgE antibody regulation Specific areas of research include the establishment of human T cell clones, their immunological, biochemical and genetical characterization and the study of cellular interactions

Applications, including a detailed curriculum vitae and naming academic referees, should be sent to **Dr Kurt Blaser, Ph D, SIAF, Obere Strasse 22, CH-7270 Davos, Switzerland**

(W6677)A

DEPARTMENT OF CLINICAL CHEMISTRY UNIVERSITY OF OULU

(WORLD HEALTH ORGANIZATION COLLABORATING CENTRE FOR RESEARCH IN HUMAN REPRODUCTION)

POSITIONS FOR SENIOR RESEARCHERS

The Department of Clinical Chemistry, University of Oulu, Finland, has been designated as a WHO Collaborating Centre for Research in Human Reproduction The laboratory facilities will be enlarged during 1990, and the Centre will act as a training centre for the WHO Special Programme of Research, Development and Research Training in Human Reproduction The central theme in all its activities will be to train scientists from developing countries in reproduction research and the necessary technologies

The Centre in Oulu should master at least the following technologies

- production of monoclonal and polyclonal antibodies, including mass production of monoclonal antibodies
- purification, modification and derivatization of monoclonal antibodies
- development and use of assay methods for steroids, peptides and proteins
- immunohistochemical methods
- techniques of molecular biology
- external and internal quality control of hormone assays

The researchers to be employed should be prepared to work at the bench and perform the necessary administrative duties They may also be needed as temporary advisers for WHO, which may necessitate travelling With a view to assembling such a research group, applications are invited for the following temporary positions

- Assistant Director (annual gross salary FIM 140 000–215 000)
- Two Senior Scientists (FIM 125 000–180 000)
- Scientist (FIM 93 000–135 000)

The assistant director and two senior scientists should hold a Ph D or an equivalent degree and have a substantial record of publications The assistant director should also have experience in leading scientific research The scientist should preferably hold a Ph D or an equivalent degree, but applications from persons with a lower degree are not excluded

Applications should include

1) curriculum vitae
2) list of publications 1980–1989
3) reprints of publications 1985–1989
4) description of the applicant's research interests, especially in the field of reproduction research
5) date when the applicant is available

The appointments are to the end of 1992 The activities of the Centre will be evaluated at four year intervals, when a decision will be taken as to their continuation

Applications should be addressed **Professor Reijo Vihko, Department of Clinical Chemistry, University of Oulu (World Health Organization Collaborating Centre for Research in Human Reproduction) Oulu, SF-90220 Oulu, Finland** (W6676)A

UNIVERSITY OF CAMBRIDGE

POST-DOCTORAL POSITION IN HUMAN MOLECULAR GENETICS

Funded by the Wellcome Trust

Applications are invited for a Post-Doctoral Fellow in molecular genetics to work on human metabolic disease The position is funded for five years and will entail participation in a group primarily concerned with the identification of candidate cDNAs involved in the development of iron storage disorders Pulsed-field gel mapping studies are already underway to identify the disease predisposing locus

Salary range £14,169 to £16,665 per annum depending on age

Applicants, who should have demonstrated their ability to carry out experimental work in a branch of molecular biology or genetics, should send three copies of their curriculum vitae and apply for further particulars to **Professor T M Cox, University of Cambridge School of Clinical Medicine, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ** Telephone (0223) 336864

Tne University follows an equal opportunities policy (1992)A

MEDICAL RESEARCH COUNCIL

COLLABORATIVE CENTRE

The MRC Collaborative Centre was founded in 1986 to act as a link between MRC basic research units and industry At the Collaborative Centre, commercially-oriented research projects are carried out with scientific support from within the MRC and financial support from industrial sponsors The *Antibody Engineering Group* at the Centre works on projects involving the manipulation of immunoglobulins, at the DNA level, to create novel protein molecules for diagnostic and therapeutic applications We currently have two short-term (two-year) openings within this Group

SCIENTIFIC OFFICER We are seeking a scientist with experience in tissue culture techniques and ELISA assays to work on the expression of recombinant antibody genes in mammalian cells The salary will be on the Civil Service Scientific Officer scale which rises to a maximum of £13,738 (inclusive of London Weighting) per annum, with the possibility of additional increments depending on performance to a maximum of £15,909 p a An optional pension scheme providing a 4 5% non-superannuable supplement is available Annual leave is 22 days plus public holidays and 2 5 priviledge days Applicants should possess a first degree, BTEC higher degree, or equivalent qualification

Please telephone for an application form, quoting reference no CC/AE SO1, on 01 959-3666 × 2270, or write to **Ms Frances Griffiths, Personnel Officer, National Institute for Medical Research, The Ridgeway, Mill Hill, London, NW7 1AA**

POSTDOCTORAL SCIENTIST We are interested in someone with experience in protein structure prediction and modelling to assist in the design of novel antibody molecules The salary will be in the range £14,031-£22,119 per annum (inclusive of London Allowance) Starting salary is dependent on experience MRC pension scheme option

Applications including a C V , list of publications, and the names and addresses of two professional referees should be made to **Mr C R Russell, Administrative Manager** at the above address, quoting reference no CC/AE PD1

Informal enquiries about the work of both vacancies may be made to Dr M M Bendig on 01 906-3811

The closing date for both applications is 24th November 1989

The MRC is an Equal Opportunities Employer (1994)A

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nature

The international journal of science with 500,000 readers throughout the world seeks

A TALENTED BIOLOGIST

Essential qualifications

- A sound education in some field of biology
- A critical and lively interest in science in general
- A sense of literacy

Research experience desirable

Applications are invited from people seeking a career in the communication of science The successful applicant will join the team responsible for assessing and editing biology manuscripts, either in Washington or in London

There will be opportunities for contributing to *Nature* in other ways Research experience in neurobiology or immunology would be particularly welcome, but those with other interests should not be deterred from applying

US applicants should send their *curriculum vitae*, with a detailed account of their education, research experience and literary skills, to the

Editor, *Nature*,
1137 National Press Building,
Washington, DC 20045

by 20 November and be prepared to be interviewed in Washington shortly thereafter

Applications from elsewhere should be sent to the

Editor, *Nature*,
4 Little Essex Street, London WC2R 3LF

by 27 November (1997)A

Head of Training and Organisation

c.£20,000-£22,000

Beckenham, Kent

Wellcome is an internationally renowned pharmaceutical group engaged in the Research, Development, Manufacture and Marketing of human healthcare products.

As part of the development of the Department of International Medical Affairs, we currently seek to appoint a key individual as Head of Clinical Training and Organisation.

This new post will involve the development of training programmes for medical departments in all territories outside the US and Canada in relation to various aspects of Clinical Practice in the industry. In addition, there will be a significant involvement in resource analysis and the development of improved communication with Local Medical Departments. This position offers good opportunity for personal career development.

Working to improve the world's health

- *The Wellcome Foundation Ltd*
- *Major International Pharmaceutical Group*
- *Research Centre at Beckenham*
- *Annual Turnover > £1,250m*
- *An Equal Opportunity Employer*

Wellcome

The successful candidate will be expected to undertake significant international travel and have excellent communication skills. A scientific/medical background and pharmaceutical industry experience are required. Previous involvement in areas such as clinical research or project management would be advantageous.

In addition to an attractive salary, our excellent benefits include 5 weeks' holiday, pension and life assurance schemes, profit share, a subsidised restaurant and first class sports and social facilities.

Please send letters of application to Wendy Hamilton at

MSL Advertising, Recruitment Resources,
32 Aybrook Street, London W1M 3JL.
Please quote reference WH/171.

(2008)A

RESEARCH SCIENTIST AND ASSOCIATE

MOLECULAR AND CELLULAR BIOLOGY

Amgen, a recognized leader in the biotechnology industry, has immediate opportunities for a Research Scientist and a Research Associate to participate in the characterization, cloning and expression of hematopoietic growth factors.

Qualified candidates for the Research Scientist position must have a Ph.D. and a minimum of 2 years' postdoctoral experience in the expression of recombinant protein in mammalian and prokaryotic systems. Research Associate candidates must have a BS or MS and a minimum of one year of experience in molecular biology and tissue culture. Industry experience is highly desirable.

Amgen offers an excellent compensation and benefits package as well as a stimulating and challenging research environment.

Qualified applicants should send a C.V. or resume, including references, in confidence, to:

AMGEN, INC.
Recruitment, Dept. #333A
Amgen Center
Thousand Oaks, CA
91320-1789

(NW4512)A

Equal
Opportunity
Employer

AMGEN

European Society for Medical Oncology

ESMO FELLOWSHIPS 1990

The European Society for Medical Oncology (ESMO) offers **5 Fellowships** to outstanding young European medical oncologists. The two year-fellowships (30,000 Dollars per year) have to be spent in one acknowledged European institution outside the country of origin of the candidates.

Requirements for the candidates: younger than 35 years, training in medical ongology, ESMO-Membership (or a pertinent request).

Start of fellowships 1st semester 1990
Deadline for submission 1st January 1990

Candidates should send their CV, together with a letter of acceptance of the host Institution and a detailed research project (basic research or clinically related cancer research) to:

ESMO Office,
Via Soldino 22,
6903 Lugano,
Switzerland.

esmo

(W6668)E

Protein Crystallographer

Synchrotron Radiation Applied to Protein Crystallography

Synchrotron radiation is the most versatile and powerful source of x-radiation available in the world today It is being used as an essential technique in many scientific disciplines and demands the existence of large - scale scientific centres with their own research and development programmes

One of the world's leading laboratories for this work is located at Daresbury in North Cheshire where we are carrying out research, with academic and industrial colleagues, into fundamental scientific questions, including some of those associated with structural and molecular biology

The Person

The Laboratory needs a person who will take responsibility for the collection and reduction of protein crystallographic data The person appointed will make use of the existing facilities for the work at the Laboratory but will also be able to assist in their development and participate in their use for original research We are looking for someone with practical experience of protein crystallography and who approaches this technique with enthusiasm and organisation Training in the use of synchrotron radiation (if required) will be given by experienced Laboratory staff The minimum qualifications required are a degree or equivalent

Pay

The appointment will be made at the grade of Scientific Officer or Higher Scientific Officer Salaries will be in the range of £9,131 to £14,909 depending on qualifications and experience A fixed term arrangement could be made A contribution towards relocation expenses may be available

If you are interested please contact the Personnel Department for an application form quoting Ref DL/133, Science & Engineering Research Council, Daresbury Laboratory, Warrington WA4 4AD
(0925) 603467
(24 hours)

CLOSING DATE
22nd November 1989

(2005)A

Daresbury
SCIENCE & ENGINEERING
RESEARCH COUNCIL

Department of Pharmacology University College London

"New Blood" Lectureship

(replacement)

Applications are invited for a vacant "New Blood" Lectureship in Molecular and Cellular Pharmacology Candidates with strong research interests in any area falling within this heading will be considered It is anticipated that suitable candidates may be able to participate in the research activities of the imminent IRC in Molecular Cell Biology

Candidates should be aged 35 or under, and persons holding permanent University posts in the U K are ineligible Salary on the Lecturer Scale £9,816 to £20,469 plus £1,650 p a London Allowance

Enquiries and applications, (including a full curriculum vitae, brief statement of research interests and names and addresses of 3 referees) should be addressed to **Professor D A Brown, Department of Pharmacology, University College London, Gower Street, London WC1A 6BT**

Equal Opportunities Employer Tel 01–387–7050 ext 3751 (2015)A

HUGHES VISITING ASSISTANT PROFESSORSHIP

Vassar College seeks a scientist with broad training in biology with emphasis on molecular neuropharmacology, neurological effects of toxic chemicals, or environmental toxicology (i e, environmental chemistry of pollutants, biological effects of pollutants) Successful candidate will teach three courses per academic year to undergraduates Two courses will be part of the regular curriculum in biology, chemistry, or physiological psychology, the other in the applicant's speciality Ph D or near completion required This is a 3 year appointment beginning August 1990 funded by the Howard Hughes Medical Institute

Submit curriculum vitae, statement of interests, and three letters of recommendation by 1 December 1989 to **Dr N Jay Bean, Chair, Hughes Appointment Search Committee, Box 65, Vassar College, Poughkeepsie, NY 12601**

Vassar College is an Equal Opportunity/Affirmative Action Employer

(NW4516)A

University of Reading

THE QUEEN'S AWARD FOR EXPORT ACHIEVEMENT

PROFESSOR IN THE DEPARTMENT OF GEOGRAPHY
and
DIRECTOR OF THE NERC UNIT FOR THEMATIC INFORMATION SYSTEMS

Applications are invited from experienced scientists for a Professorship in the Department of Geography linked with the Directorship of the NERC Unit for Thematic Information Systems The appointment will be made from as early a date as can be arranged

The Unit for Thematic Information Systems, which is located in the Department of Geography, carries out research into techniques of spatial data processing to support investigations of the natural environment

Applicants must have sound scientific qualifications and research experience in one or more relevant fields Experience of managing major research projects and groups will be expected

Further information may be obtained from the **Registrar, Room 212, Whiteknights House, P O Box 217, The University, Whiteknights, Reading RG6 2AH** (Telephone 0734 318045)

The closing date for applications is 27 November 1989

(1973)A

The University of Manitoba

UM

INDUSTRIAL RESEARCH CHAIR IN FERMENTATION TECHNOLOGY

Applications are invited for a Senior tenured or tenure track appointment effective 1 July 1990 as Industrial Research Professor in Fermentation Technology The Chair has been established through a five year funding program from the Natural Sciences and Engineering Research Council in collaboration with an industrial sponsor The Chairholder will be expected to take the lead in developing a research program in fermentation technology Candidates must have an international status in the field and will be expected to interact in the research and development fields of ABI Biotechnology Inc

The Chairholder will be expected to attract significant research contracts from industry to augment the substantial funding for equipment, research staff, and operating expenses which are available

The applicant should have a Ph D degree and at least ten years of industrial research experience or equivalent education and experience Salary is negotiable The Chair is primarily a research position, and as such teaching loads will be light with the emphasis on graduate student teaching and research

Both women and men are encouraged to apply In accordance with Canadian Immigration requirements, priority will be given to Canadian citizens and permanent residents

Applicants should arrange to have three letters of reference, curriculum vitae, recent reprints and a brief description of present and prospective research forwarded to **P Loewen, Chairman Search Committee, Department of Microbiology, The University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2**

Applications and/or nominations will be accepted up to the 30th September 1989, or until the position is filled

(NW4520)A

BRITISH HEART FOUNDATION

LECTURER/SENIOR LECTURER BASIC SCIENTIST RESEARCH POSTS

The British Heart Foundation invites applications from post-doctoral research workers to compete for a number of basic scientist research posts (non-clinical) at lecturer or senior lecturer level The research programme need not necessarily relate to cardiology, but should relate to the cardiovascular system in its broadest sense and be undertaken in a university department within the UK

An award would be for a period of five years with the possibility of an extension of not more than a further five years The award reimburses for salary at the appropriate university scale and carries an allowance of up to £10,000 per annum for research expenses

Applicants must be UK citizens, or have worked in the UK for at least the past three years, or must have done so before taking up an appointment overseas The candidate should be aged approximately 28-33 years and should have shown outstanding ability in carrying out original and independent research

Application may be made by the head of department or the candidate with support from the head of department

Application forms and further details (quoting reference N/BS) are available from

Valerie Mason
The Research Funds Manager
British Heart Foundation
102 Gloucester Place
London
W1H 4DH

Closing date for applications 12 December 1989

(1991)A

Research Scientists
Thrombosis Research

Corvas, Inc., a biopharmaceutical company located in the biomedical research center of La Jolla, is expanding to address exciting product opportunities in the thrombosis field We are seeking outstanding scientists to join our research effort Qualified candidates will have a Ph D, prior industrial R&D experience, and skills in one of the following areas

Protein Biochemistry

Study of enzyme/substrate, ligand/receptor or protein/protein interactions Analysis of protein structure and function by molecular modeling

Bio-Organic Chemistry/Drug Design

Design and syntheses of novel anti-thrombotic agents Experience in peptide, peptidyl mimetic chemistry, and computer-aided design is required

Corvas offers attractive salaries and benefits, an excellent opportunity for equity participation and a highly interactive scientific environment For consideration, please send letter of interest and CV to **Human Resources Director, Corvas, Inc, 11077 North Torrey Pines Road, La Jolla, CA 92037**

Corvas, Inc.

(NW4522)A

Laborleiter Diabetes/Stoffwechsel

Byk Gulden ist ein deutsches Unternehmen der forschenden pharmazeutischen Industrie mit weltweiten Aktivitäten

Für unsere Forschung und Entwicklung suchen wir einen jungen promovierten Biochemiker oder Biologen mit experimenteller Erfahrung auf den Gebieten Stoffwechselregulation, Zellbiologie und Endokrinologie

Wenn Sie

- moderne analytische Methoden zur Untersuchung der Wirkungen neuer Substanzen sowohl auf zellularer und subzellularer Ebene als auch an Organen und geeigneten Versuchstieren beherrschen,
- Verständnis für chemische/strukturelle Sachverhalte mitbringen,
- mit dem PC umgehen können,
- fließend englisch sprechen und schreiben und
- gerne im Team arbeiten,

würden wir uns über Ihre Bewerbung freuen

Bitte senden Sie Ihre kompletten Unterlagen (Lebenslauf, Foto, Zeugnisse) unter Angabe Ihrer Gehaltsvorstellung und des möglichen Eintrittstermines an unsere Personalabteilung SPA

Wir antworten umgehen

BYK

Byk Gulden Pharmazeutika
Byk-Gulden-Straße 2
7750 Konstanz

(W6679)A

FULL-TIME POSITION AS

ASSISTANT RESEARCH CELL and DEVELOPMENTAL BIOLOGIST

PhD in Molecular Biology/Cell Biology

Must have experience in muscle cell culture and in analysis of cell and protein turnover, growth factor cloning, expression, isolation, and characterization, receptor binding, extracellular matrix components isolation and characterization The Candidate will be in charge of a program to define growth factor interactions with matrix components in vitro and in vivo The job is guaranteed for one year only with a possibility for renewal for three years, depending on grant funding Salary range $37,700-$47,100 depending on experience Closing date December 7, 1989 Write to **Dr Richard Strohman, Department of Molecular and Cell Biology, Division of Cell & Developmental Biology, University of California, 351 Life Sciences Addition, Berkeley, CA 94720** The University of California is an equal opportunity, affirmative action employer (NW4528)A

UNIVERSITY OF NEWCASTLE UPON TYNE

Department of Medicine

JUNIOR RESEARCH ASSOCIATE

A Junior Research Associate is required to join a group studying autoimmune thyroid disease The post is funded by a two year MRC project grant on the Grade 1B salary scale £9,816-£12,381 per annum

The project is entitled "The role of TSH receptors on orbital fibroblasts in thyroid associated ophthalmopathy" The post would suit a graduate with a degree in Biochemistry, an interest in Immunology and experience of cell culture would be helpful, but are not essential The work would be suitable for submission for an M Phil degree

Further information is available from Professor Kendall-Taylor Tel 091 2325131 Ext 24635 or bleep 2154 or Dr Perros Tel 091 2226000 Ext 7012 Applications should include a full curriculum vitae together with the names and addresses of 3 referees and should be sent to **Professor Kendall-Taylor, Department of Medicine, Medical School, The University, Newcastle upon Tyne, NE2 4HH** by 17th November 1989

Department of Biochemistry and Genetics

RESEARCH ASSOCIATE

Applications are invited for a one year MRC funded research associate post at either postgraduate or postdoctoral level The project will involve the use of techniques such as site-directed mutagenesis to investigate the main immunogenic region of the nicotinic acetylcoline receptor Experience in recombinant DNA techniques would be an advantage

Salary will be up to £12,381 per annum on either the Grade 1A or 1B salary scale according to qualifications and experience

Applications, including curriculum vitae and the names of three referees should be sent as soon as possible to **Dr N S Millar, Department of Biochemistry and Genetics, The Medical School, The University, Newcastle upon Tyne, NE2 4HH** (Tel 091 2227423)

Sir James Knott

POSTDOCTORAL FELLOWSHIPS

Applications are invited for two Sir James Knott Fellowships, tenable for two years from 1 October 1990 The Fellowships, tenable in any Faculty, are open to persons who appear likely to make a noteworthy contribution to knowledge One of the Fellowships will be reserved for candidates who have not graduated from this University The other will be open to any person, but other things being equal, will be awarded to one who has not taken a first degree as a member of this University The value of each Fellowship is £10,458 in the first year and £11,088 in the second year

Further particulars and application forms (which must be returned by 30 November 1989) may be obtained from the **Registrar (Fellowships), The University, Newcastle upon Tyne NE1 7RU** (1983)A

PHLS CENTRE FOR APPLIED MICROBIOLOGY AND RESEARCH

Division of Pathology

RESEARCH/PRODUCTION ASSISTANT

Required for expanding production team working with immunodeficency viruses The duties of the post are varied and will involve applied research in addition to routine production work Applicants should possess an honours degree in a relevant biological science The post will be graded at Basic Grade Microbiologist

The post is for a fixed term of 2 years, the salary range is £8,255–£11,073 per annum (under review)

National Health Service terms and conditions will apply

Application forms can be obtained from the Personnel Officer, PHLS Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wilts, SP4 0JG Tel Idmiston (0980) 610391 ext 240 to whom completed forms should be returned by 23rd November 1989 quoting post number 0521 (2000)A

PHLS CENTRE FOR APPLIED MICROBIOLOGY AND RESEARCH

DIVISION OF BIOLOGICS

Research Assistant

Is required for the Bacterial Antigens Group to join a team commencing a project concerning the development of a vaccine against group B Neisseria Meningitidis The post is funded by the MRC and is for 1 year initially The duties of the post are to assist in research into the mechanisms of pathogenesis of the meningococcus Applicants should posses either an honours degree in a relevant biological science or a recognised technical qualification, or have suitable technical experience The post will be graded at either Basic Grade Microbiologist or MPT II depending on the qualifications of the successful applicant

Salary (under review) will be in the range £7,378 to £11,824

National Health Service Terms and Conditions will apply

Application forms can be obtained from the Personnel Officer, PHLS Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wilts SP4 0JG Tel (0980) 610391 Ext 240, to whom completed forms should be returned by 23rd November 1989 quoting Post Number 0571

UNIVERSITY OF LONDON, BIRKBECK COLLEGE

SERC CASE AWARD

Applications are invited for this research award between the Chemistry Department, Birkbeck College and the Division of Biotechnology, PHLS Centre for Applied Microbiology and Research

The project is concerned with the molecular basis of gold resistance in bacteria Research training will be provided in areas of chemistry, biochemistry and biotechnology, including the isolation of bacteria and proteins, protein and DNA sequencing and structural studies using high frequency NMR

Enquiries can be made to Dr Peter Sadler, Department of Chemistry, Birkbeck College, University of London, 29 Gordon Square, London WC12H 0PP (Telephone 01 380 7480, or to Dr Michael Scawen, Division of Biotechnology, Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire, SP4 0JG, Tel 0980 610391

Applicants should possess a first or upper second class honours degree Applications (including a Curriculum Vitae and the names and addresses of two referees) should be sent to Dr Sadler at Birkbeck College as soon as possible

The basic SERC grant will be supplemented by research merits awards (2004)A

UNIVERSITY OF CAMBRIDGE

Department of Botany

POST-DOCTORAL RESEARCH ASSISTANT

Applications are invited for a post doctoral research assistant to wor on a project supported by th S E R C Membrane Initiative, in volving the study of ion channel from plant cells incorporated int artificial planar lipid bilayers Applications are invited fron candidates with experience in ior transport/electrophysiology, o with experience in the isolation o membrane proteins, but who wisl to gain experience of electro physiology In either case th experience need not be with plan cells

The post is available from January 1990, for about 2½ years on the 1A scale The start date negotiable, but should be before June 1990

Informal enquiries and applica tions including a curriculum vita and the names and addresses o two referees should be sent a soon as possible to Professo E A C MacRobbie, Botany School University of Cambridge, Downin Street, Cambridge CB2 3EA (Tel 0223 333943) (1998)A

POSTDOCTORAL POSITION

available immediately, to participate in ongoing study of excitation-secretion coupling in endothelial cells Techniques employed include patch clamp and fluorescent Ca^{2+} indicators Ph D with experience in electrophysiology required Salary in $20,000-$30,000 range commensurate with experience Send CV and names of two references to **Drs D Adams or C van Breemen, Dept of Pharmacology R189, University of Miami School of Medicine, P O Box 016189, Miami, FL 33101** AA/EOE (NW4500)A

DIRECTOR OF SURGICAL RESEARCH

The Mary Imogene Bassett Hospital, affiliated with Columbia University, is seeking an academic general surgeon or general surgeon/subspecialist with research experience to expand the surgical research program, conduct laboratory research, and supervise surgical resident research projects Adequate space and initial support are available Responsibilities will be divided evenly between clinical activities and The Mary Imogene Bassett Research Institute Submit inquiries and CV to **Michael S Gold, MD, Surgeon-in-Chief, The Mary Imogene Bassett Hospital, Atwell Road, Cooperstown, NY, 13326 Telephone (607) 547-3968** (NW4515)A

DIRECTOR
RADIATION THERAPY

Roswell Park Cancer Institute, affiliated with the State University of New York at Buffalo School of Medicine and Biomedical Sciences, seeks to fill the position of Director of Radiation Therapy The successful applicant will direct an active department of Radiation Oncology in a hospital renowned for patient care and cancer research The successful applicant must be board certified in Radiation Therapy and have proven capabilities in patient care, teaching, research and administration Curriculum vitae should be submitted to

Andrew A Gage M.D.
Deputy Institute Director
Roswell Park Cancer Institute
Elm and Carlton Streets
Buffalo, New York 14263

ROSWELL PARK
MEMORIAL INSTITUTE

Roswell Park is an Affirmative Action Employer
(NW4525)A

ASSISTANT/ASSOCIATE PROFESSOR IN IMMUNOLOGY

The Immunology unit of The Toronto Hospital Arthritis Center and the Department of Immunology, University of Toronto wish to recruit an assistant or associate professor to join a multidisciplinary research group with interest in the cellular and molecular aspects of the immune response We seek to bring together molecular biology, cellular immunology, microbiology and immunogenetics in the study of the pathogenesis of immunological diseases Applications are invited from researchers with Ph D or M D degrees with appropriate post-doctoral research training

Canadian immigration guidelines require that consideration in the first instance be given to Canadian citizens and permanent residents of Canada

Inquiries and applications (curriculum vitae and names of three references) should be addressed to **Dr R D Irman, Departments of Medicine and Immunology, Toronto Western Hospital, 399 Bathurst Street, Toronto, Ontario, Canada M5T 2S8 FAX (416) 369–6477**
(NW4519)A

POSTDOCTORAL FELLOWS

POSTDOCTORAL FELLOWS in Molecular Biology/Applied Computer Science are available at the Dana-Farber Cancer Institute and the Biostatistics Department of the Harvard School of Public Health Candidates must have a recent Ph D in molecular biology, molecular genetics or computer science The research will center on the problems of comparative analysis and idendification of genetic sequence patterns This will involve collaboration with active experimental molecular biologists and computer software developers One position is available immediately and one anticipated for April, 1, 1990 Send vitae and 3 letters of reference to **Dr Temple F Smith, Dana-Farber Cancer Institute, 44 Binney St , Room 1154, Boston, MA 02115** An Affirmative Action/Equal Opportunity Employer (NW4508)A

UNIVERSITY OF LEICESTER
BIOINFORMATICS

Under an EEC-sponsored twinning arrangement between the Department of Anatomy, University of Leicester, U K and the Department of Medical Biochemistry, Aarhus University, Denmark, the assembly of a database of protein characteristics and immuno-localisation patterns is being undertaken

A post is available for a citizen of an E E C member state (other than the United Kingdom) as a Research Assistant in the University of Leicester Applications should be graduates, fluent in written and spoken English and skilled in computing and/or microscopy To implement the link between the laboratories, staff will have to meet frequently and the appointee will enjoy extensive scientific visits to the Danish Laboratory for which an appropriate expenses budget is available A suitable candidate may be entitled to register for a higher degree of the University of Leicester The post is of 3 years' duration, it is available immediately but may be held open for a particularly strong candidate

The salary will be on the Research IB scale (£9,816-£12,381 p a) and is superannuable Informal enquiries and applications should be directed to either **Dr C D Ockleford, University of Leicester Medical School, University Road, Leicester LE1 9HN, U K (Tel 0533 523021, FAX 0533 523031)** or **Professor J E Celis, Institute of Medical Biochemistry, Ole Worms Alle, Building 170, University Park, Aarhus University, DK-8000 Aarhus C, Denmark (Tel + 45 86 12 93 99, FAX 45 86 13 11 60)** (2012)A

FACULTY POSITION
ANALYTICAL MICROSCOPIST/ MATERIALS SCIENTIST

Applicants are sought for a full time tenure-track position at the assistant professor level A strong background in analytical electron microscopy and a PhD in materials science or a related discipline is required Candidates should be interested in teaching undergraduate and graduate courses and in developing a strong research program Proposed starting date is negotiable

Northwestern is an Affirmative Action/Equal Opportunity Employer Hiring is contingent upon eligibility to work in the United States Send resume, publications and the names of three references to

Professor L D Marks,
Northwestern University,
Department of Materials Science
and Engineering,
Evanston, IL 60208, USA. (NW4526)A

TWO POSTDOCTORAL POSITIONS

in protein crystallography available July 1990 (1) X-ray structure determination and simulation of flavoproteins of clinical interest (2) Study of dynamics in protein crystals using MD simulations and X-ray refinement by simulated annealing Experience in crystallography preferred, but candidates with background in biochemistry or computational chemistry with a desire for training in crystallography will also be considered

Send resume to **Dr John Kuriyan, Laboratory of Molecular Biophysics, The Rockefeller University, 1230 York Avenue, New York, NY 10021**

An equal opportunity/affirmative action employer
(NW4523)A

UNIVERSITY OF SOUTHAMPTON

Department of Biochemistry

POSTDOCTORAL POSITION

An SERC funded postdoctoral position is available in the laboratory of Dr B Connolly The research involves the study of protein nucleic acid interactions using the restriction endonuclease and modification methylase Eco RV enzymes It is hoped to delineate the mechanism by which the two enzymes achieve catalysis at specific DNA sequences Among the approaches being used are preparation of modified bases for incorporation into oligonucleotides, oligonucleotide synthesis, protein chemistry, enzymology, site directed mutagenesis and other molecular biology techniques Candidates should have a Ph D and experience in some of these areas The post is available immediately for a three year period, but can be delayed to 1 April 1990

Salary will be between £10,458 and £12,381 per annum, depending on age and experience

Applicants should send a C V and the names and addresses of two referees to **Dr Connolly, Department of Biochemistry, University of Southampton, Southampton, SO9 3TU, U K**, by 23 November 1989 *Please quote reference number 117/JAD/jm* Informal enquiries can be made by telephone (0703–594330) to Dr Connolly (1978)A

University of Glasgow

Department of Pharmacology

Postgraduate Research Assistant

required for one year on a project on adenosine receptors in the nervous system Some experience with electrophysiological techniques or general pharmacological smooth muscle preparation would be an advantage but is not essential It may be possible for the person appointed to register for a higher degree

Starting date to be agreed at a salary of £9,816 per annum, on Research Assistant 1B scale

Applications should be sent to **Professor T W Stone, Department of Pharmacology, University of Glasgow, Glasgow, G12 8QQ** by 30th November, 1989 (2001)A

Postdoctoral Position — Molecular Biology of Oncogenes

NIH/NRC position available for recent Ph D with training in molecular biology to study the structure/function characteristics of nuclear oncogene (see PNAS **86**, 1186, 1989)

Position renewable for one year with starting salary of $28,000 Must be U S citizen Send C V and names of three references to **Dr Herbert C Morse III, Laboratory of Immunopathology, Building 7, Room 302, N I M, Bethesda, MD 20892**

The NIH is an equal opportunity employer (NW4457)A

THE UNIVERSITY OF MELBOURNE

Australia

ICI — MASSON CHAIR OF CHEMISTRY

Applications are invited for the ICI — Masson Chair of Chemistry This is a new Chair created as a result of the recommendations of a recent external review of the School of Chemistry The Chair represents a significant partnership in academic-industrial collaboration between ICI Australia and the University of Melbourne

The appointee will be a distinguished chemist with an international reputation, who will provide leadership in all areas of the School The appointee will be expected to take over the Headship of the School for a period of five years in the first instance In particular, it is anticipated that the new Professor will have the ability to manage the important change of the School from what is currently a federation of three academic Departments to a single School within which the three basic disciplines can flourish The new Chair will be backed by an effective administrative support structure

The base salary is A$63,919 per annum The University is currently examining the question of salary loadings and responsibility allowances

Further information about the position, application procedure, superannuation, travel and removal expenses, housing assistance and conditions of appointment is available from the Registrar, or from Appointments (37087), Association of Commonwealth Universities, 36 Gordon Square, London WC1H OPF All correspondence (marked "Confidential") should be addressed to the Registrar, The University of Melbourne, Parkville, Victoria 3052, Australia (tel [03] 344 7528 Facsimile 344 6897)

Applications close on 15 December 1989

The Council reserves to itself the right to make no appointment or to fill the Chair by invitation at any stage

An equal opportunity employer (W6661)A

INSTRUCTOR-IN-RESIDENCE

Position available immediately

Applicants should have a Ph D with a minimum of 1 year postdoctoral experience and a strong background in lipid biochemistry

Send C V and 3 references to **Dr D L Cinti, Dept Pharmacology, UConn Health Center, Farmington, CT 06032**

An Affirmative Action Equal/Opportunity Employer M/W/H (NW4490)A

CENTRAL BIRMINGHAM HEALTH AUTHORITY IN ASSOCIATION WITH UNIVERSITY OF BIRMINGHAM

WOLFSON RESEARCH LABORATORIES

QUEEN ELIZABETH MEDICAL CENTRE

SENIOR RESEARCH SCIENTIST

(Molecular Biology)

A post doctoral Research Scientist is required to join the Molecular Biology group in the Wolfson Research Laboratories Current work is focused on the development of molecular biological technologies for clinical diagnosis and the application of genetic engineering techniques to modify antibodies and pollen allergens Experience of DNA cloning, sequencing, site-directed mutagenesis and familiarity with hybridisation and polymerase chain reaction is desirable The post involves supervision of associated research staff The post is funded by the Department of Health and is available for 3 years in the first instance

Salary is within the range £11,778–£15,394, under review (Senior Biochemist scale with Whitley Council Conditions)

Candidates are invited to contact Dr M R Walker on 021 472 1311, ext 4544 to arrange a visit to the laboratory

Job description and application form available from Personnel Department, Queen Elizabeth Hospital, Edgbaston, Birmingham B15 2TH Tel 021 472 1311, ext 3003 Job Ref 473

Closing date 23rd November 1989

The Authority is Committed to Equal Opportunities in Employment (1995)A

University of Bristol

CHAIR IN INORGANIC CHEMISTRY

Applications are invited for the Chair in Inorganic Chemistry which will fall vacant on the retirement of Professor F G A Stone, FRS

Suitably qualified candidates are invited to submit applications by 15th December, 1989 Further particulars of the appointment may be obtained from the **Registrar, University of Bristol, Senate House, Bristol BS8 1TH** (1965)A

Duke University

2 tenure track positions — Geophysicist Asst or assoc prof level, Hydrogeologist Asst prof Send c v, statement of research interests and names of 4 referees by Dec 15 **Search Committee, Dept of Geology, Duke Univ, Durham, NC 27706 USA** (NW4412)A

AGRICULTURAL AND FOOD RESEARCH COUNCIL

INSTITUTE OF ANIMAL PHYSIOLOGY & GENETICS RESEARCH

CAMBRIDGE RESEARCH STATION

Babraham, Cambridge CB2 4AT

RESEARCH ASSISTANT

required to work on signal tran
duction pathways in T-lymph
cytes The post will give goc
opportunities to become famili
with a wide range of techniques
cell biology and biochemistr
Previous experience in cell-cultu
would be beneficial but not esse
tial

The appointment will be mac
at Scientific Officer level (Salai
£9131-12223) or Higher Scientif
Officer level (Salary £1067
14909) depending on experienc
and qualifications, but candidate
should have a First or Uppe
Second Class honours degree in
relevant subject Non-contribu
tory superannuation scheme 22 c
25 days leave

Application forms can be ob
tained from The Personnel Off
cer, quoting Ref MVI/P by 2
Nov 1989 (1987)A

A position is available in macromolecular structure determination using crystallography or NMR at assistant to full professor level, entry level applicants are encouraged Appointment is through the Institute of Molecular Biology in the Departments of Chemistry and Biology NMR Facility and the Multiwire Area X-ray Diffractometer Facility are available for use Please send resume with research proposal and have three letters of recommendation sent to **R H Kretsinger, Chair, Search Committee, Department of Biology, University of Virginia, Charlottesville, VA 22901** *Equal Opportunity/Affirmative Action Employer* (NW4478)A

POSTDOCTORAL POSITION

available immediately to study purine nucleoside phosphorylase and G-CSF retroviral-mediated gene transfer in vivo Experience in molecular biology and/or cell biology desirable

Please send curriculum vitae and names of three references to **Bill Osborne, Ph D, Department of Pediatrics, RD-20, University of Washington School of Medicine, Seattle, WA 98195, or telephone 206–543–4735** (NW4511)A

TWO POSTDOCTORAL POSITIONS

in protein crystallography available July 1990 (1) X-ray structure determination and simulation of flavoproteins of clinical interest (2) Study of dynamics in protein crystals using MD simulations and X-ray refinement by simulated annealing Experience in crystallography preferred, but candidates with background in biochemistry or computational chemistry with a desire for training in crystallography will also be considered

Send resume to **Dr. John Kuriyan, Laboratory of Molecular Biophysics, The Rockefeller University, 1230 York Avenue, New York, NY 10021.**

An equal opportunity/affirmative action employer

(NW4523)A

Queen Mary College (University of London)
Department of Chemistry

Postdoctoral Research Assistant

Applications are invited immediately for a postdoctoral position funded by a joint SERC/DTI initiative called "Silicon Towards 2000"

The work will involve the preparation of metal-containing volatile precursors, these will then be used to modify the properties of porous silicon, produced electrochemically Experience in handling volatile air-sensitive compounds and/or the synthesis of organometallic compounds would be a particular advantage

Remuneration and general conditions of employment will be those appropriate to a Research Assistant Grade 1A, with a starting salary in the range £13,500–£15,000 (including London Allowance)

For further details, please contact

Professor B J Aylett
Department of Chemistry
Queen Mary & Westfield College
Mile End Road
London E1 4NS

(2014)A

FACULTY POSITION IN CELL BIOLOGY
CARNEGIE MELLON UNIVERSITY

Applications are invited for a tenure-track position in the Department of Biological Sciences at Carnegie Mellon University Applicants must be strongly motivated toward carrying out research in cell biology Candidates who are just completing their postdoctoral research training will be considered for a position of starting assistant professor, exceptionally well-qualified individuals may be considered for appointment at a higher level

To apply, send curriculum vitae and a concise description of research interests (no more than three pages), and, in addition, have three letters of recommendation sent to **Dr John Woodford, Chair, Cell Biology Search Committee, Department of Biological Sciences, Carnegie Mellon University, 4400 Fifth Avenue, Pittsburgh, PA 15213** Deadline for applications 15 December 1989

Carnegie Mellon University is an Equal Opportunity/Affirmative Action Employer

(NW4487)A

MOLECULAR BIOLOGISTS

The Department of Biological Sciences is strengthening a group in molecular biology and is expanding its faculty at the **ASSISTANT/ ASSOCIATE PROFESSOR** level Applications from individuals in all areas of molecular biology are encouraged, but the department has particular interests in cell-cell interaction, signal transduction, growth regulation, gene expression, and microbial genetics There is ample opportunity for interaction with existing faculty in molecular and developmental genetics of both eukaryotic and prokaryotic organisms Generous start-up funds and competitive salaries are offered Send curriculum vitae, selected reprints, and names and addresses of three referees by 1 December 1989 to **Dr M R Felder, Molecular Biology, Search Committee, Department of Biological Sciences, University of South Carolina, Columbia, SC 29208**

Equal Opportunity/Affirmative Action Employer

(NW4502)A

PAC BIO

SENIOR SCIENTISTS
SCIENTISTS
RESEARCH ASSOCIATES

Pacific Biotechnology Ltd (Pac Bio) is an expanding biopharmaceutical company involved in the development and manufacture of human therapeutics using the techniques of modern molecular biology

Applications are invited from Senior Scientists (relevant experience required), Scientists (PhD required) and Research Associates (degree or technical qualifications required) who have experience in one of the following areas

ENDOCRINOLOGY
IMMUNOLOGY
PROTEIN CHEMISTRY
MOLECULAR AND CELLULAR BIOLOGY
ANIMAL PHYSIOLOGIST

Pac Bio uses state of the art technology and has strong international links with academic research groups and with other biotechnology companies The company offers a challenging and dynamic research environment, competitive salary and equity participation

Written applications, including curriculum vitae and details of previous experience, with the names of two referees, should be directed to

The Chief Scientific Officer
Pacific Biotechnology Ltd
P O Box 172, Kings Cross NSW 2011, Australia
Telephone (02) 332 3513 Fax (02) 332 1581

(W6681)A

CHARLES STEWART MOTT DISTINGUISHED PROFESSOR OF SUSTAINABLE AGRICULTURE

Michigan State University College of Agriculture and Natural Resources requests applications and nominations for the Charles Stewart Mott Distinguished Professorship of Sustainable Agriculture This endowed chair will be responsible for establishing innovative programs and catalyzing a campus-wide program of excellence in sustainable agriculture The position has research, teaching and extension responsibilities, with a charge to enrich education programs with a philosophy of sustainable development, conduct research designed to impact agriculture through integration of alternative, traditional and biotechnological management systems, develop programs to design, test and disseminate agricultural technology, and enhance issues of sustainability and service to international agriculture programs

MSU is searching for a scientist and leader with a distinguished record of achievement and interdisciplinary orientation Candidates will need to possess significant research stature and organizational skills We are seeking an agricultural scientist, broadly trained in agronomy, with the ability to interact with faculty from a diverse array of disciplines The position will be tenured in the Department of Crop and Soil Science

Applications for the Charles Stewart Mott Distinguished Professorship of Sustainable Agriculture will be received until January 10, 1990, or until a suitable candidate is identified Applications, nominations and questions should be forwarded to **Dr George W Bird, Chair, Charles Stewart Mott Professor of Sustainable Agric Search Comm, Dept of Entomology, Michigan State Univ, East Lansing, MI 48824, 517-353 8133, Fax 517-353 4354, Telex 650 2247573 MIC**

(NW4494)A

RESEARCH SCIENTIST — IMMUNOLOGIST

DNAX Research Institute, located near Stanford University, has established strong basic research programs combining recent advances in immunology and molecular biology in an academic environment

A staff scientist position is available for an individual with a doctorate degree in an immunological field, and a minimum of three years experience in the generation of antigen specific human T cell clones, antigen processing, T cell activation, and lymphokine production The successful candidate will participate in a continuing program to investigate regulation of allergic specific human IgE synthesis Demonstrated organizational skills and supervisory experience are also required

Salary range $48K-$55K

Responses must be postmarked not later than November 26 1989, to be considered

Interested candidates should sent a curriculum vitae, representative publications, a statement of research interests, and names of three references to

DNAX RESEARCH INSTITUTE
Human Resources Department
Attention P. Cooke
901 California Avenue, Palo Alto, CA 94304-1104

AN EQUAL OPPORTUNITY EMPLOYER M/F/H

(NW4498)A

UNIVERSITY OF BRITISH COLUMBIA

Department of Microbiology

Molecular Immunologist

The Department of Microbiology, University of British Columbia invites applications from molecular immunologists for an assistant professor level, tenure track position The individual we seek will have a Ph D postdoctoral experience a strong research record and show potential for developing an internationally recognized research program which will complement ongoing programs in immunology, virology and bacterial pathogenosis Salary will be commensurate with qualifications and experience The successful candidate will be expected to participate in the Department's large and expanding Ph D progarm In accordance with Canadian immigration requirements priority will be given to Canadian citizens and permanent residents of Canada Closing date for applications is February 28 1990 Position will be available July 1, 1990 The position is subject to final budgetary approval

Please send curriculum vitae a summary of research interests and the names of three referees to The Head Department of Microbiology, University of British Columbia, #300 6174 University Boulevard, Vancouver, B C V6T 1W5

The University of British Columbia is committed to the Federal Government s employment equity program and encourages applications from all qualified individuals

(NW4517)A

UNIVERSITY OF NEWCASTLE UPON TYNE

THE MEDICAL SCHOOL

LECTURESHIPS (2)

DEPARTMENT OF PHYSIOLOGICAL SCIENCES

Applications are invited for two Lectureships (one of five years fixed-term) in the Department of Physiological Sciences Physiological Sciences, together with the Departments of Clinical Biochemistry and Microbiology in the Faculty of Medicine and the Department of Biochemistry and Genetics in the Faculty of Science, form the Cross Faculty School of Biomedical & Biomolecular Sciences The Department is responsible for teaching an Honours Science course in Physiological Sciences, and Physiology in the integrated medical curriculum and to dental students

Applications will be welcomed from suitably qualified candidates in any area of physiology, including cellular and molecular physiology The newly appointed lecturer will be expected to pursue a vigorous research programme and good opportunities exist for modern research in an interdisciplinary environment

The appointments will be made to the Lecturers' Grade A scale (£10,458–£15,372 p a) or Grade B scale £16,024–£20,469 p a) In the case of a medically qualified person, the appointment will be made to the Lecturers' Pre Clinical General Grade (£12,879–£20,874 p a) or to the Lecturers Pre Clinical Higher Grade (£18,690–£21,489 p a) Starting salaries will be according to qualifications and experience

Further particulars may be obtained from the Senior Assistant Registrar, Establishments, The University 6 Kensington Terrace Newcastle upon Tyne, NE1 7RU, with whom applications (3 copies) together with the names and addresses of three referees, should be lodged not later than 4th December 1989 (2013)A

ROWETT RESEARCH INSTITUTE

BUCKSBURN, ABERDEEN, AB2 9SB

VACANCIES IN PROBIOTICS RESEARCH

The Rowett Research Institute is Western Europe's leading centre for studies in nutritional science It is located on the outskirts of Aberdeen and has close links with other prominent educational and research centres in Grampian Region which is one of Scotland's most thriving and attractive areas

A multidisciplinary research group is being established at the Institute to investigate modes of action of probiotics The programme, which is funded by a consortium of companies and the DTI, will run for three years The research group will consist of 6 scientists, together with support staff appointed specifically for this project This group will work in collaboration with a number of existing Institute staff whose expertise is particularly relevant

CELL BIOLOGIST/HISTOLOGIST/ ELECTRON MICROSCOPIST

As the cell biologist in this group you will use microscopic techniques to study microbial binding to mucosal receptors and the effects of dietary and other factors on binding You will work with a biochemist and a microbiologist studying the interactions both *in vivo* and *in vitro* between lectins, glycoproteins and polysaccharides of both dietary and microbial origin, with mucosal receptors of the small intestine and the modulation of binding by dietary sugars and lectins

You should have relevant experience in cell biology and histology and be familiar with modern cytochemical techniques using both light and electron microscopy

MICROBIOLOGIST/IMMUNOLIGIST

As one of two microbiologists appointed to this group you will have responsibility for in vivo microbiology, assessing microbial activity in the digestive tract, evaluating the probiotic effects of indigenous and administered microorganisms You will also have responsibility for studying the immunological aspects of probiosis, assessing the effects of the enteric microflora on the humoral and cell-mediated immune systems

As a candidate for either of these posts you should be self motivated, a good communicator and able to work effectively in a multidisciplinary team Both appointments will be made either at the Scientific Officer or Higher Scientific Officer grade

Salary Range — (SO) £10,678 to £12,223, (HSO) £10,678 to £14,909

Minimum Qualifications — Appointees to the SO grade should have a 1st or upper 2nd class honours degree with at least 2 years relevant post graduate experience for HSO grading

PhD STUDENTSHIP (CHEMIST/BIOCHEMIST)

A PhD studentship is also offered as part of the project, to examine in vitro the sugar specificities of the adhesins of selected potential probiotics and of pathogenic species

This studentship will be administered in accordance with the normal terms of Research Council's studentships except that the maintenance grant will be augmented to £4,500 p a Candidates will be expected to have a 1st or upper 2nd honours degree

Applications for these posts are invited from suitably qualified candidates who should write with full details including a CV and the names and addresses of 2 referees to the Secretary of the Institute Informal enquiries can be made by telephone to Dr Malcolm Fuller, ext 332, or Dr Andrew Chesson, ext 320, both on Aberdeen (0224) 712751

Closing date 24th November 1989 (2010)A

UNITED MEDICAL AND DENTAL SCHOOLS

Division of Anatomy, Guy's Campus

LECTURESHIP IN MOLECULAR NEUROBIOLOGY

Applicants for this post should have experience in molecular biological techniques and an interest in neurobiology The lecturer will be expected to pursue independent research and contribute to undergraduate teaching The appointment will be for 5 years in the first instance Salary on University non clinical Lecturer Scale A £10,458–£15 372 Scale B £16 014–£20 469 plus £1,650 London Weighting Allowance

To discuss the post informally contact Professor M Berry (01 955 4360) or Dr Andrew Lumsden (01 955 4413) For further details phone 01–928 9292 ext 3341 Applications in the form of full curriculum vitae (6 copies) should be sent to the Personnel Officer, UMDS, St Thomas's Campus, Lambeth Palace Road, London SE1 7EH quoting reference G/ANA/419 by 14 November 1989 (1888)A

DIRECTOR OF HIGHER EDUCATION AFFAIRS

Swindon, Wilts

The Natural Environment Research Council wishes to appoint a senior person to be responsible for the formulation and implementation of policies in support of higher education with relevance to environmental research This support includes the provision of research grants, post-graduate training awards and fellowships tenable in higher education institutions, together with scientific services and facilities The total value of NERC's contribution to these areas exceeds £23 million a year The post-holder will play an important role in developing and maintaining links and relations between NERC research activities and the higher education sector, and will be responsible for the effective operation of NERC's peer review system, and for establishing and maintaining detailed overviews of departmental and individual performances

The successful applicant will have a good awareness of current higher education sector activities in the sciences relevant to environmental research, and is likely to be working at present at professorial level in a university or polytechnic The appointment will be for a three year period in the first instance, with the possibility of eventual permanent appointment The post would be ideally suited to a secondment from the higher education sector

Salary will normally be in the range of £30,001 to £33,991 per annum, but a higher salary may be available for a candidate with special qualifications or experience.

A non-contributory pension scheme is available, along with a generous annual leave allowance

The post will be located at NERC's Headquarters in Swindon, Wiltshire, and is likely to require a significant amount of travel throughout the UK Assistance towards relocation expenses may be payable in certain circumstances

Application forms can be obtained from June Shields, Personnel Section, Natural Environment Research Council, Polaris House, North Star Avenue, Swindon, Wilts SN2 1EU Tel 0793 411695

Completed application forms including a full CV to be returned by 24th November 1989

The Natural Environment Research Council is an equal opportunities employer

Natural
Environment
Research
Council

(2020)A

POSTDOCTORAL POSITION

immediately available for Associate Research Scientist with recent Ph D Will work in laboratory studying gene expression and cell differentiation in T cells and human thymocytes Knowledge of tissue culture, molecular biology or cellular biology desirable Willing to train Please submit cv and names of three references to **Joan Harmon, Personnel Dept, NYU Medical Center, 550 First Ave, New York, NY 10016**

An Equal Opportunity Employer M/F (NW4509)A

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nature

the widest
international
selection of jobs
in science
—EVERY WEEK

U S Department of Energy

ALEXANDER HOLLAENDER DISTINGUISHED POSTDOCTORAL FELLOWSHIP PROGRAM

Hollaender Postdoctoral Fellowships provide research opportunities in the life, biomedical, and environmental sciences Fellowships are tenable at various Department of Energy and university laboratories with programs supportive of the Office of Health and Environmental Research Stipend is $35,000 A doctoral degree received after April 1, 1988, is required as is U S citizenship or PRA status Deadline is January 16, 1990

For applications contact **Hollaender Postdoctoral Fellowships, Science/Engineering Education Division, Oak Ridge Associated Universities, P O Box 117, Oak Ridge, TN 37831-0117, (615) 576-3190** (NW4167)E

MRC LABORATORY OF MOLECULAR BIOLOGY

The following PhD projects are available for studies commencing in October 1990 A limited number of MRC studentships, subject to the usual eligibility requirements, will be offered on a competitive basis These studentships can be supplemented by up to £500 p a for students with additional laboratory responsibilities

Dr B Barrell	Development of computer programs to analyse large DNA sequences
Dr A Bloomer	Crystallographic studies of protein assemblies
Dr P Evans	Crystallographic studies of allosteric and catalytic mechanisms of phosphofructokinase
Dr R Henderson	Structural analysis of photochemical intermediates and mutants of bacteriorhodopsin
Dr R Kay	Molecular basis of intercellular signalling controlling cell differentiation and patterning in *Dictyostelium*
Dr J Kendrick-Jones	Probing the role of myosin type 'motors' in cell motility using protein engineering, cell biological and biochemical approaches
Dr J Kilmartin	Biochemistry and genetics of yeast mitosis
Dr G Koch	Structural studies on luminal ER proteins
Dr P Lawrence	Developmental genetics of *Drosophila*
Dr A Leslie	Structural studies of mutants of chloramphenicol acetyltransferase
Dr A McLachlan	Maximum entropy in crystallography Structure and sequence analysis of proteins
Dr B Pearse	Coated vesicles, adaptors and sorting
Dr H Pelham	Targeting of proteins to their correct locations in yeast and animal cells
Dr T Rabbitts	Studies of developmentally regulated oncogenes
Dr D Rhodes	Structural studies on DNA-binding proteins and their interactions with DNA
Dr A Smith	Site-directed modification of germ-line immunoglobulin genes by homologous recombination
Dr M Stewart	Molecular interactions and assembly dynamics of nuclear envelope components studied using directed mutagenesis and expression of cDNA
Dr A Tomlinson	Molecular genetics of *Drosophila* eye development
Dr A Travers	Nucleic acid structure and biological function
Dr J Walker	Genetic and structural studies of mitochondrial respiratory enzyme complexes
Dr J White	Cellular differentiation during the early development of *Caenorhabditis elegans*
Dr M Wilcox	Molecular and genetic analysis of *Drosophila* integrins
Dr D Zimmern	Studies on the function and expression of the proteins and ribozymes of two RNA viruses

For further information, applicants should write directly to Dr A A Travers, MRC Laboratory of Molecular Biology, Hills Road, Cambridge, CB2 2QH

MRC
Medical Research Council

IRC Centre for Protein Engineering An Interdisciplinary Research Centre (IRC) for Protein Engineering has been set up under MRC administration, with premises close to the MRC Laboratory of Molecular Biology, and also in Cambridge University Studentships will be available in protein engineering studies, ranging from molecular genetics to high field NMR and X-ray crystallography The studentships can be held in IRC, MRC or University laboratories Further information will be available after 1 January 1990, and candidates should then contact **Prof A R Fersht, Department of Chemistry, Lensfield Road, Cambridge CB2 1EW, or Dr G Winter, MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH**

(2018)A

Plymouth Marine Laboratory

BIOLOGIST/BIOCHEMIST

Salary up to £14,909

A scientist is required for a three year period to investigate the bioavailability, uptake and accumulation by estuarine oligochaetes of heavy metals from sediments

Candidates should have a good honours degree and a minimum of 2 years' post-graduate research experience in a relevant field Salary will be in the range £10,678 to £14,909 per annum Conditions of service include a generous holiday allowance and a non-contributory pension scheme

Write for further details and an application form to Mrs Carol Addison, NERC, Plymouth Marine Laboratory, Citadel Hill, Plymouth PL1 2PB, quoting post No 6000 Closing date for completed applications is 16th November 1989

The Natural Environment Research Council is an equal opportunity employer

**Natural
Environment
Research
Council**

(2019)A

PHLS CENTRE FOR APPLIED MICROBIOLOGY AND RESEARCH

Division of Biologics

RESEARCH SCIENTIST

A position is available in the Bacterial Antigens Group for a new project, funded for 3 years concerning the isolation and purification of proteins from certai pathogenic bacterial and their use in immunological detection tests

This position would suit a recently qualified PhD Experience in protein purification techniques would be advantageous The post would be graded at Basic or Senior Grade Microbiologist depending on experience and qualifications

Salary (under review for 1989 award) will be in the range of £7378 (BGM) to £15,394 (SGM)

Further information can be obtained from Dr A Robinson, tel (0980) 610391 ext 2585

National Health Service terms and conditions will apply

Application forms can be obtained from the Personnel Officer, PHLS Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wilts, SP4 0JG Tel (0980) 610329 ext 240 to whom completed forms should be returned by 23rd November 1989 quoting Post Number 0569 (1999)A

International Research Fellowship Opportunities

for U.S and Foreign Postdoctoral Scientists

*Senior International Fellowships

These fellowships offer opportunities to U S biomedical, behavioral, or health scientists to conduct research in a foreign institution The program is for scientists who have established themselves in their chosen career in the United States and whose professional stature is well-recognized by their peers and institution officials

The purpose of this program is to enhance the exchange of ideas and information about the latest advances in the health sciences, basic, clinical, and public health and to permit U S scientists to participate abroad in ongoing study or research in foreign institutions

Fellowships are awarded for a period of 3 to 12 months and provide stipend, travel, and living allowance

*International Research Fellowships

These fellowships offer opportunities to foreign scientists in the formative stage of their career to extend their research experience in U S laboratories Selections are first made by the Nominating Committee in participating countries, regions, or areas Over 50 countries or regions in the Americas, Africa, Europe, Asia and the Far East, plus Australia, and New Zealand participate in the program

The purpose of this program is to forge relationships between distinguished scientists in the United States and qualified scientists in other countries in order to study health-related problems of mutual interest

Fellowships are awarded for a minimum of 12 months and provide stipend, travel, and institutional allowance

*Foreign-Supported Fellowships

These fellowships for U S scientists are supported by specific foreign countries and areas They provide opportunities for scientists to conduct collaborative research in the country that provides funding

The purpose of this program is to enhance the exchange of research experience and information in the biomedical, behavioral, and health sciences The maximum period of support for all programs is 1 year, and the minimum period of support varies with each program

Participating countries and areas are FINLAND, FRANCE, FEDERAL REPUBLIC OF GERMANY, IRELAND, ISRAEL, JAPAN, NORWAY, SWEDEN, SWITZERLAND, and TAIWAN

**Health Scientist Exchanges

These programs for U S scientists support short- and long-term visits to AUSTRIA, BULGARIA, HUNGARY, POLAND, ROMANIA, the SOVIET UNION, and YUGOSLAVIA

The purpose of these programs is to conduct collaborative activities in one of the health sciences or the health-related fields that are of mutual benefit to the United States and the participating country Priority is given to visits designed to strengthen or expand ongoing collaborative relationships or to explore prospects for long-term cooperation

The financial provisions include round-trip travel and in-country costs

*AIDS Fellowships

There are two programs to support scientists interested in AIDS research The postdoctoral fellowship program supports U S and foreign scientists to conduct research abroad and in the United States, respectively The minimum support is for 3 months, and the maximum support is for 24 months Institutional awards have been made to four U S institutions for a 5-year period

The epidemiology training grants provide long-term training which may lead to the M S or Ph D degree and short-term training in the United States or participating foreign countries Only foreign scientists from developing countries may participate in this program Institutional awards have been made to ten U S institutions

The financial provisions of these programs include stipend, travel, and a host institutional allowance

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*Requests for additional information on Senior International, International Research, Foreign-Supported Fellowships, and AIDS Fellowships should be sent to International Research and Awards Branch, John E Fogarty International Center, National Institutes of Health, Bethesda, MD 20892

**Requests for additional information on Health Scientist Exchanges should be sent to International Coordination and Liaison Branch, John E Fogarty International Center, National Institutes of Health, Bethesda, MD 20892

(NW4477)E

CENTRAL BIRMINGHA
HEALTH AUTHORITY

TOP GRADE
CYTOGENETICIST
with Greater Responsibility

Required to head the Cytogenet Unit at Birmingham Matern Hospital

Applicants are expected have extensive experience in Cy genetics

For further information and arrange an informal visit plea contact Mrs Anne Green, Direc of Clinical Chemistry, 021 4 4851 ext 6102

For an application form and j description please contact Mr Bramma, Senior Personnel Offic District Offices, Central Birmin ham Health Authority, Vince Drive, Edgbaston, Birmingha B15 2TZ Tel 021 472 2294 e 3703 Job reference no DHQ4 89

Closing date 30th Novemb 1989

The Authority is Committed Equal Opportunities in Emplo ment (1996)A

THE UNIVERSITY OF LEEDS
Department of Chemical Pathology
and Immunology

LECTURER (NON CLINICAL) (NAAS)

Applications are invited for the above post which is available immediately

Candidates should have a PhD in biochemistry, immunology or a related subject, together with suitable postdoctoral experience and will be expected to establish a research team working in the application of molecular biology, biochemistry or immunology to medicine

Salary on the scale for Lecturer Grade A (£10,458–£15,372) or exceptionally Grade B (£16,014–£20,469) according to qualifications and relevant experience

Informal enquiries may be made to Professor Whicher (tel 0532 335673)

Application forms and further particulars may be obtained from and completed applications forwarded to the **Registrar, The University, Leeds LS2 9JT** (tel 0532 333969) quoting reference no 84/24 *Closing date for applications 22nd December 1989*

(1946)A

MISCELLANEOUS

IN AUTO IMMUNE disease,if th disease commences as a singl event affecting a single cell even affecting a single cell and propa gates by positive feedback it i likely that the immune reactio releases more antigen —Reply t Box No 1968J c/o Nature, 4 Little Essex Street, London WC2R 3LF (1968)J

NATO ADVANCED STUDY INSTITUTE

"THE APPLICATION OF CHARGE DENSITY RESEARCH TO CHEMISTRY AND DRUG DESIGN"

17-27 April 1990

Costa Brava, SPAIN

The aim is to instruct in the methods of Charge-Density analysis by X-ray and neutron diffraction and to explore the use of this information in chemistry and drug design

Information and application forms can be obtained from the directors

G A Jeffrey
Department of Crystallography,
University of Pittsburgh,
Pittsburgh, PA 15260, U S.A.
FAX 412-624-1882 TELEX 199126
PHONE 412-624-9300

J.F Piniella
Department de Cristallografia,
Universitat Autónoma de Barcelona,
08193 Bellaterra, Barcelona, SPAIN
FAX 34-3-581-2003 TELEX 52040
PHONE 34-3-581-1609

The number of participants is limited to 80 Applicants are advised to apply as soon as possible (W6682)C

Royal Postgraduate Medical School
(University of London)

In Situ Hybridisation Course

23rd–27th April 1990

This one week course provides an introduction to the most important concepts and procedures of *in situ* hybridisation The course consists of a lecture programme designed to illustrate the theoretical aspects and recent applications in different fields, including pathology, virology and neurobiology There will be a practical session which will cover all aspects of the technique

Topics covered will include

- Construction and labelling of single and double stranded RNA and DNA probes with radiolabels and biotinylated nucleotides
- Tissue handling and collection, including cytological preparations
- Fixative choice and tissue processing
- Pre-hybridisation treatments and probe choice
- Optimisation of conditions for hybridisation and post-hybridisation stringency washes
- Visualisation systems using autoradiography or chomogenic methods
- Quantification of reaction product
- Analysis of results and troubleshooting

Course organisers	Professor Julia M Polak, Dr S J Gibson and Dr G Terenghi (Histochemistry)
Course fee (inc catering)	£400 full course (inc practicals) £240 lectures only
Further details and application forms from	Wolfson Conference Centre Royal Postgraduate Medical School Hammersmith Hospital Du Cane Road London W12 0NN
Telephone 01 740 3117	
Closing date for applications	15 January 1990

(2016)C

THE AMERICAN ASSOCIATION FOR CANCER RESEARCH ANNOUNCES

AACR Special Conference in Cancer Research

STEROID RECEPTORS, TRANSCRIPTION FACTORS, AND GENE EXPRESSION

February 10–13, 1990
Catamaran Resort Hotel, San Diego, California

PROGRAM COMMITTEE
Bert W O'Malley, Chairperson
Ronald M Evans Marc E Lippman

SCIENTIFIC PROGRAM

OVERVIEW
I Herskowitz, San Francisco, CA
W McGuire, San Antonio, TX

STEROID HORMONE RECEPTORS
R Evans, San Diego, CA
B O'Malley, Houston, TX
P Chambon, Strasbourg, France
H Samuels, New York, NY

TRANSCRIPTION FACTORS
R Roeder, New York, NY
R Tjian, Berkeley, CA
L Guarente, Cambridge, MA
A Greenleaf, Durham, NC

RECEPTORS AND GENE EXPRESSION
M Beato, Marburg, FRG
J -A Gustafsson, Huddinge, Sweden
P Mellon, La Jolla, CA
D Shapiro, Urbana, IL

GENE TRANSCRIPTION AND DEVELOPMENT
M Green, Cambridge, MA
A Wolffe, Bethesda, MD
V Pirrotta, Houston, TX
M Rosenfeld, La Jolla, CA

SIGNALS FOR TRANSCRIPTIONAL CONTROL
J Baxter, San Francisco, CA
G Schutz, Heidelberg, FRG
R Kornberg, Stanford, CA
M Tsai, Houston, TX

ONCOGENES, HORMONES AND CANCER
E Ziff, New York, NY
B Groner, Basel, Switzerland
I Verma, San Diego, CA
M Lippman, Washington, DC
B Katzenellenbogen, Urbana, IL

INFORMATION AND APPLICATION FORMS
American Association for Cancer Research
330 Market Street, 2nd Floor
Philadelphia, PA 19106
215–440–9300 215–440–9313 (FAX)

APPLICATION DEADLINE DECEMBER 11, 1989
Late applications will be accepted on a space-available basis (NW4389)C

Royal Postgraduate Medical School

(University of London)

Immunolabelling for Electron Microscopy

8th–12th January 1990

A one-week course which will include lectures and practicals

Speakers include.

- Julian Beesley
- John Priestley
- Brian Causton
- Giselle Hodges
- Bharat Jasani
- Geoffrey Newman
- David Hopwood

The course will cover.

Preparation and processing of tissue
Pre- and post-embedding immunohistochemistry
Colloidal gold techniques
Cryoultramicrotomy
Multiple staining procedures

Course organisers

Dr C Sarraf (Histopathology)
Professor J M Polak (Histochemistry)
Dr A Merighi (Histochemistry)

Course fee (inc catering) £350 00

Further details and application forms from
Wolfson Conference Centre
Royal Postgraduate Medical School
Hammersmith Hospital
Du Cane Road
London W12 0NN
Telephone 01-740 3117 (1979)C

MOLECULAR ENDOCRINOLOGY AND TECHNIQUES FOR HORMONE ACTION

February 18–22, 1990

This course will consist of lectures in methodology with comprehensive syllabus covering receptors, gene transcription, mRNA and protein synthesis, recombinant DNA, protein hormones and cyclic nucleotides

Co-directors M R Hughes and B W O'Malley
Fee $365 ($245 fellows and students)
For further information contact **Bonnie Cuthbert, Department of Cell Biology, Baylor College of Medicine (713)-798-6200**
DEADLINE—February 2, 1990 (NW4144)C

STUDENTSHIPS

ST MARY'S HOSPITAL MEDICAL SCHOOL

(a constituent College of Imperial College of Science, Technology and Medicine)
(University of London)
Norfolk Place, LONDON W2 1PG

DEPARTMENT OF PHARMACOLOGY AND TOXICOLOGY

PhD STUDENTSHIP

Available immediately for 3 years in the above department to join an existing team studying the fate of nitrogen-containing compounds and their relationship to human disease

Applications, in the form of a full C V with names and addresses of 2 referees should be submitted as soon as possible to **Dr S C Mitchell** at the above address (1962)F

RESEARCH STUDENTSHIP IN ANIMAL BEHAVIOUR

Applications are invited for a postgraduate scholarship to study behaviour in poultry The scholarship is tenable for 3 years, is funded by the British Egg Marketing Board Research Education Trust and will lead towards a Ph D degree The subject will be The effect of housing on spatial and fear behaviour in laying hens

The research will be carried out mainly at the Edinburgh Research Station of the AFRC Institute of Animal Physiology and Genetics Research with Dr B O Hughes and Dr M C Appleby and form part of a major resea programme into farm ani physiology, behaviour and v fare Some field work may ta place at the West of Scotla College, Ayr

Applicants should posses a Firs Upper Second Class Hono Degree in Zoology, Agriculture a related subject and should sen CV, together with the names two referees to Dr B O Hugh AFRC Institute of Animal Phy ology and Genetics Resear Roslin, Midlothian EH25 9 Closing date November 30, 198

(2002)

CONGRESS

ISPMB

Announcing

the Third Congress of the

INTERNATIONAL SOCIET FOR PLANT MOLECULAR BIOLOGY

October 6-12, 1991
Tucson, Arizona
USA

TUCSON '91

Members will receive details by January, 1990
Non-members may request information by writing to:

ISPMB
Woo Wester Conference Consultants
2934½ Beverly Glen Circle, Suite 383
Los Angeles, CA 90077 USA

(NW4475)

UNIVERSITY OF CAMBRIDGE

Postdoctoral research fellowship Molecular biology of glial cells

This is a three year fellowship, funded by the MRC The project is to use recombinant DNA and ribozyme technology to alter the expression of genes for cell surface adhesion molecules, proteases and protease inhibitors in glial cell lines, and assay for effects on *in vitro* and *in vivo* models of axon regeneration Candidates should be trained in molecular biological techniques

For further information call J Rogers or J Fawcett on (0223) 333899

Send written applications, including c v and the names of 2 referees to - **Dr J Rogers, Physiological Laboratory, Downing St, Cambridge CB2 3EG, England** (1964)E

Ministerio de Educación y Ciencia
Comisión Interministerial de Ciencia y Tecnología

Research in Spain.

The Spanish Ministry of Education and Science is encouraging foreign national researchers, by means of grants/scholarships, to spend time in Spain to pursue research projects in universities and research centers.

Grant announcement for Ph.D's who have read their doctoral dissertation within the last seven years:

Application deadline date May 31, 1990
Beginning date Last quarter of 1990,
Exceptionally, other dates may be considered
Grant duration Six months to two years

Grant announcement for researchers on sabbatical leave:

Application deadline date December 31, 1989
Grant duration Three to twelve months

INFORMATION AND APPLICATION FORMS:

(W6674)H

AWARDS

THE 1990 LOUISA GROSS HORWITZ PRIZE

The Louisa Gross Horwitz Prize was established under the will of the late S Gross Horwitz through a bequest to Columbia University, and is named to honor the donor's mother Louisa Gross Horwitz was the daughter of Dr Samuel David Gross (1805–1889), a prominent surgeon of Philadelphia, and author of the outstanding "Systems of Surgery" who served as president of the American Medical Association

Each year, since its inception in 1967, Columbia University has awarded The Louisa Gross Horwitz Prize for outstanding basic research in the fields of **Biology or Biochemistry** The purpose of this award is to honor a scientific investigator, or group of investigators, whose contributions to knowledge in either of these fields is deemed worthy of special recognition

The prize consists of an honorarium and a citation which are awarded at a special presentation event Unless otherwise recommended by the Prize Committee, the prize is awarded annually The 1989 awardees are Dr Alfred G Gilman of the University of Texas, Southwestern Medical Center School at Dallas and Dr Edwin G Krebs of the Howard Hughes Medical Institute Research Laboratories, University of Washington School of Medicine in Seattle

QUALIFICATIONS FOR THE AWARD

The Prize Committee recognizes no geographical limitations The prize may be awarded to an individual or a group When the prize is awarded to a group, the honorarium will be divided among the recipients, but each member will receive a citation Preference will be given to work done in the recent past

Prospective recipients should be nominated to the Chairman of the Horwitz Prize Committee, Dr Samuel C Silverstein Nomination letters should include

1 A summary, preferably less than 500 words, of the research on which this nomination is based
2 A summary, preferably less than 500 words, of the significance of this research in the fields of biology or biochemistry
3 A brief biographical sketch of the nominee, including positions held and awards received by the nominee
4 A listing of up to ten of the nominee's most significant publications relating to the research noted under Item 1
5 A copy of the nominee's curriculum vitae

Send to Dr Samuel C Silverstein, Department of Physiology & Cellular Biophysics, Columbia University, 630 West 168th Street, New York, New York 10032

Deadline for receipt of nominations is January 4, 1990 (NW4482)N

THE MICHELSON-MORLEY AWARD

The prestigious Michelson-Morley Award is presented by Case Institute of Technology to recognize the distinctive achievements of a particular scientist or engineer The award is named in honor of Albert A Michelson, first professor of physics at Case Institute and 1907 Nobel laureate, and Edward W Morley, distinguished professor of chemistry at Western Reserve College Joint research by Professors Michelson and Morley is highlighted by their experiments related to the velocity of light These scientific probes prepared the way for the discoveries of Albert Einstein and decisively advanced modern theoretical physics

The stated purpose of the award is to honor the individual chosen for eminent contributions to knowledge and to the well-being of mankind, to perpetuate the tradition of scholarly inquiry exemplified by the independent and collaborative research of Professor Michelson and Morley, and to call to the attention of the general public the continuing contributions of the scientist and engineer to human welfare Deadline for nominations 15 November 1989

Nominations should be sent to **Glenn R Brown, Dean of the College, Case Western Reserve University, Crawford Hall #7100, 10900 Euclid Avenue, Cleveland, OH 44106** (NW4486)N

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nature

the widest international selection of jobs in science — EVERY WEEK

POST-DOCTORAL FELLOWSHIPS AVAILABLE

National Institutes of Health

Post-doctoral positions are available immediately in the Section on Molecular Neurobiology at the National Institute on Alcohol Abuse and Alcoholism for studies on the mechanisms of receptor-effector coupling (J Biol Chem 264 11754, 1989), the structure-function relationships of G-protein linked and ion channel receptors using receptor gene expression and site-directed mutagenesis (J Biol Chem 264 9266, 1989, Biochem Pharmacol 38 1197, 1989), and mechanisms of receptor regulation Candidates should have a strong background in receptor pharmacology/ biochemistry, molecular biology or electrophysiology Starting salaries will be commensurate with experience ($25,000-$28,000) For IRTA Fellowships, candidates must be U S citizens with less than 3 years post-doctoral experience For Fogarty Fellowships, candidates must be non-U S citizens with less than 3 years post-doctoral experience

Send C V and the names of three references to

Dr Claire M Fraser, Chief
Section on Molecular Neurobiology
Laboratory of Physiologic and Pharmacologic Studies
National Institute on Alcohol Abuse and Alcoholism
12501 Washington Avenue
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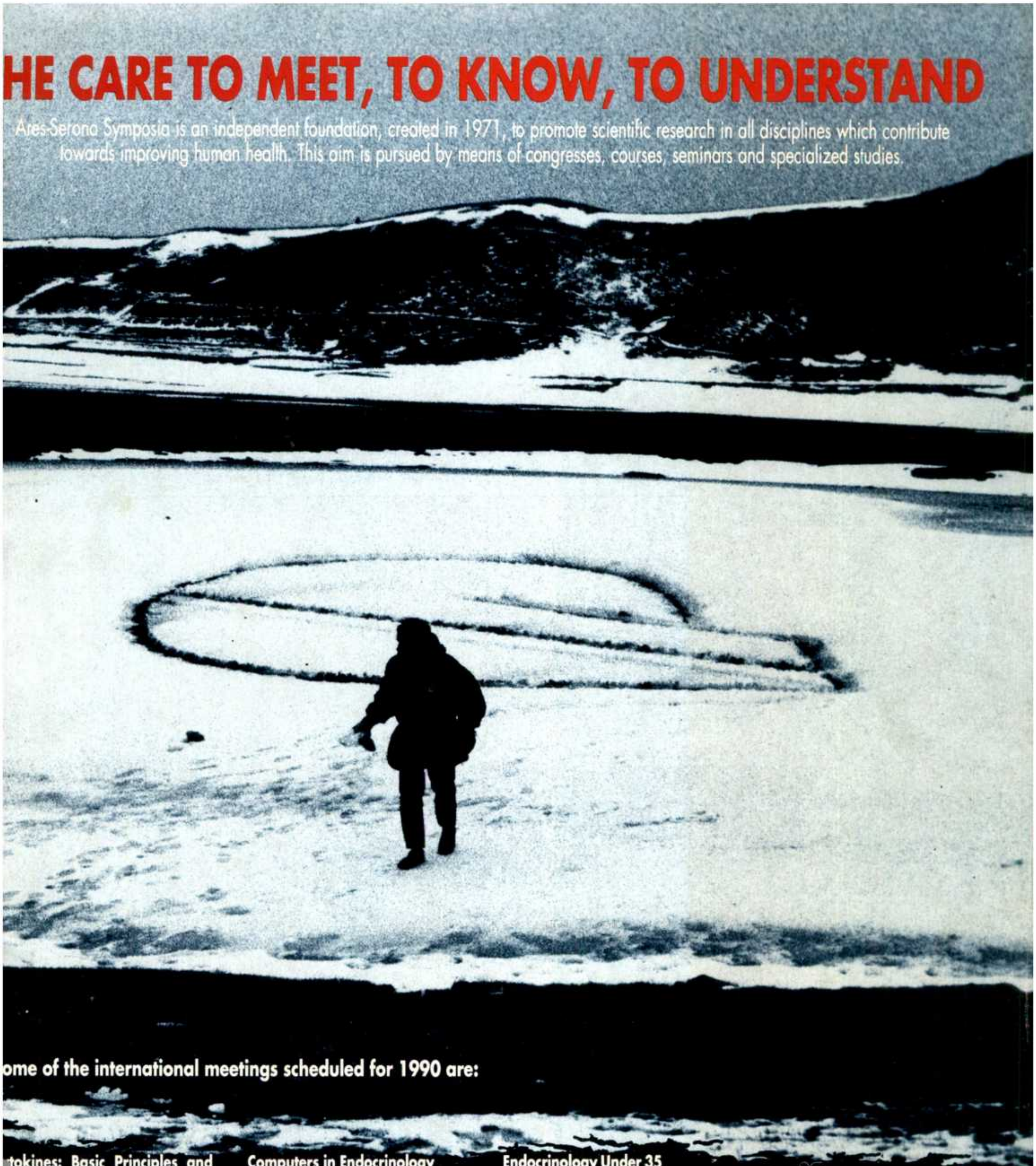

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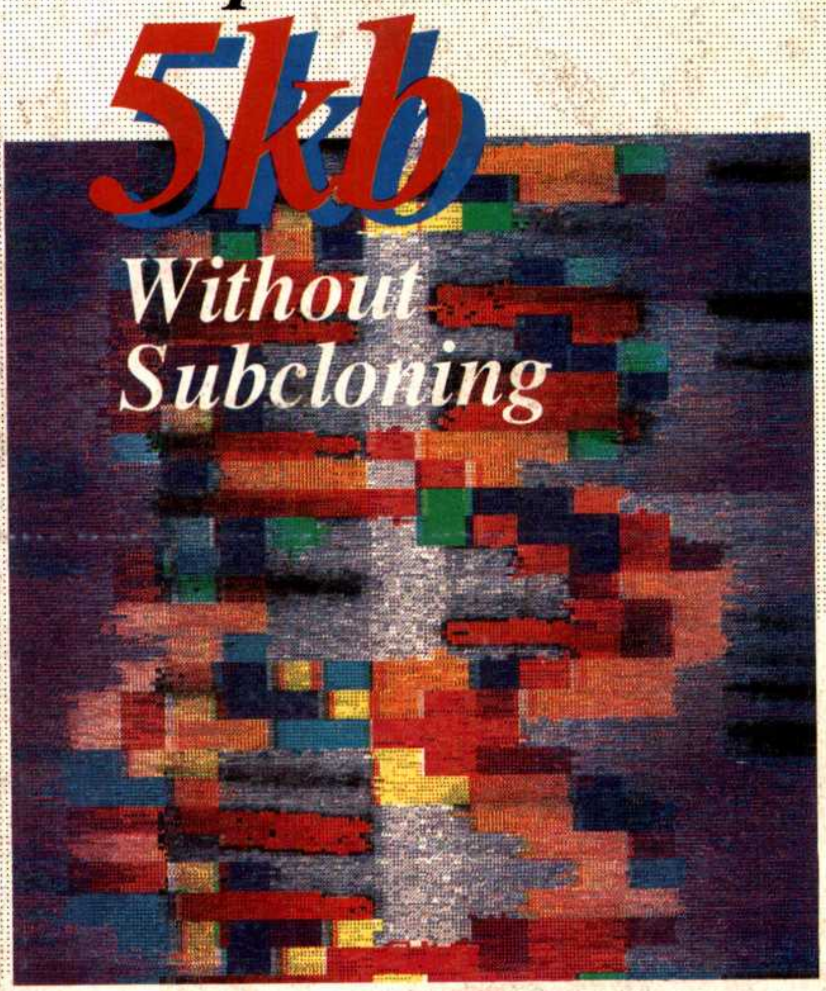

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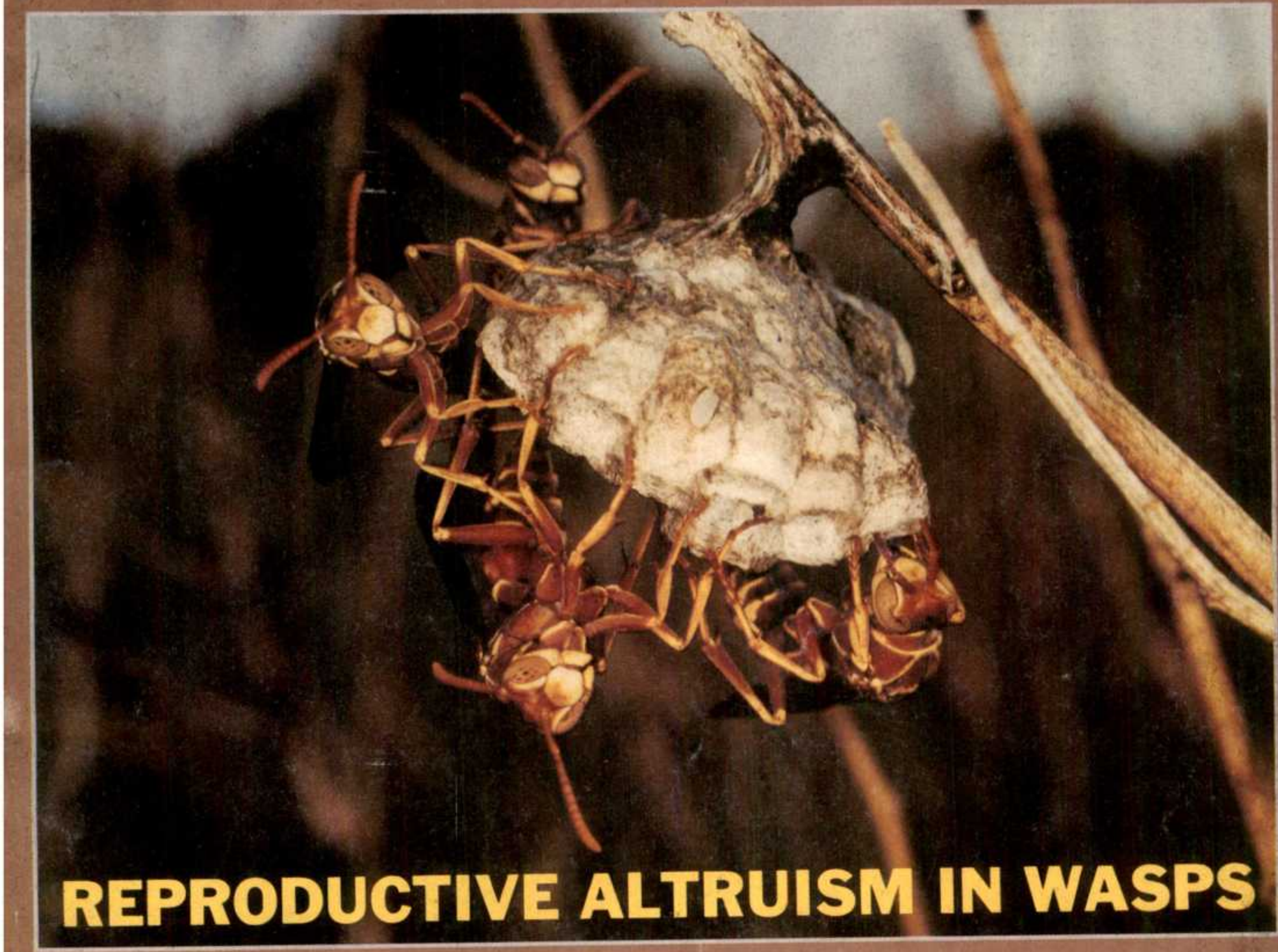

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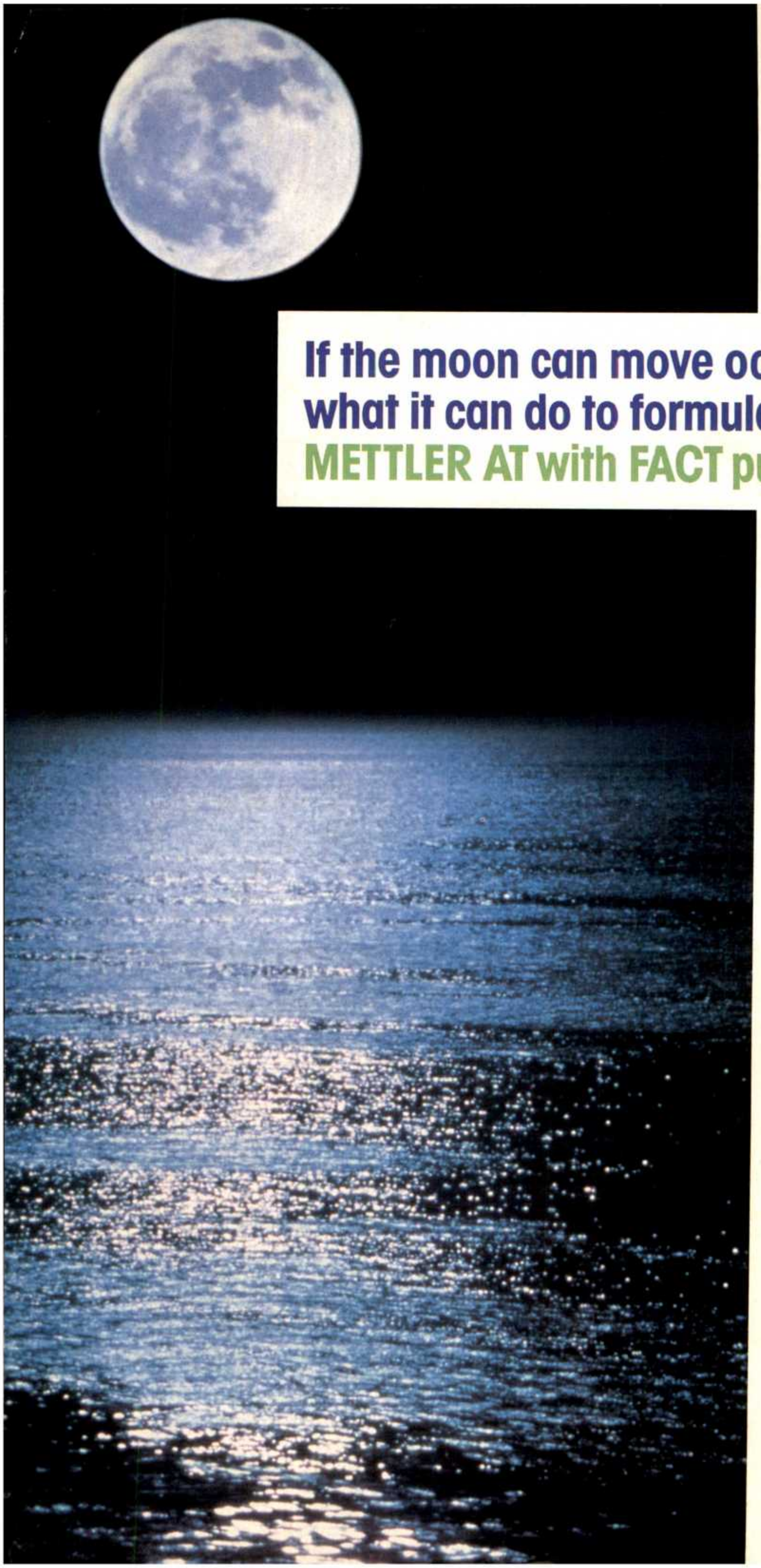

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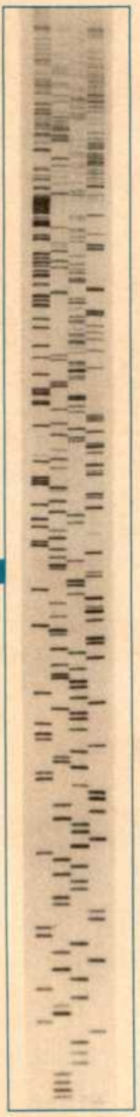

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nature

16 November 1989

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◀ Social insects like these female *Polistes bellicosus* wasps reproduce indirectly — most individuals achieve genetic success by helping to rear offspring of colony mates. But in species not divided into morphological castes, sacrifices are made only for rather close relatives. See page 268. Autumn Books, pages 303–326.

THIS WEEK

Ozone hole spreads

Airborne measurements taken during August and September 1987 show that there was high-latitude ozone loss outside the Antarctic ozone hole. So not only is the geographic extent of the ozone loss larger than generally thought, but ozone is lost earlier in the year than previously reported, pages 233 and 225.

Second-hand gene?

The structure of the *E. coli* 'chaperonin' protein PapD, which prevents pilin subunits from aggregation and degradation prior to their assembly into pili at the outer membrane, shows it to comprise two domains, each with the characteristic immunoglobulin fold. Together with sequence similarity to a lymphocyte differentiation antigen this suggests *E. coli* may have acquired *PapD* by gene transfer from a eukaryotic host. Pages 248 and 224.

Extraterrestrial primer

Did extraterrestrial organic molecules play a role in prebiotic evolution on Earth? On page 255, E. Anders calculates that a substantial amount of organic matter from comets and asteroids did survive atmospheric entry to reach the Earth's surface, primarily carried by small particles associated with the last stage of the period of heavy meteoritic bombardment.

Building bridges

Methods of increasing protein stability without reducing enzymatic activity will make it possible to use enzymes in a wider range of industrial processes. On page 291 it is shown that the melting temperature of a protein, T4 lysozyme, can be increased by as much as 23°C when three additional disulphide bonds are introduced by site-directed mutagenesis.

No link to depression

Further analysis of the Amish pedigree in which linkage of manic depression to a locus on chromosome 11 has been reported reduces the evidence for linkage to below statistical significance. Pages 238 and 222.

Tunnel vision

Negative differential conductivity, until recently seen only in macroscopic electronic devices such as tunnel diodes, can be obtained on an atomic level by use of a scanning tunnelling microscope, page 258. The recent achievement of large-scale integration of STMs on a silicon wafer suggests that the microscopic tunnel diode could be incorporated in a practical device.

Attractive proposition

The discovery of galactic streaming motions of up to 600 kilometres per second towards the constellations of Hydra and Centaurus led to the suggestion of a 'Great Attractor', a concentration of galaxies causing the motions. A new survey of galaxies in this region fuels the debate on large-scale motions, page 251.

Taking the strain

Radiotelemetry has been used to monitor the strain on the limb bones of gibbons in their natural habitat, the forest canopy. The data reveal greatly reduced bending and compressive forces in comparison with other mammals, contributing to the evolution of the long slender forelimb bones of 'brachiating' mammals. Pages 270 and 229.

Crossed lines

What links deep-sea noises, thunder claps and amorphous semiconductors? See page 223.

Author Index

The Author Index to Vol. 341 follows Autumn Books. Guide to Authors faces page 1 of index.

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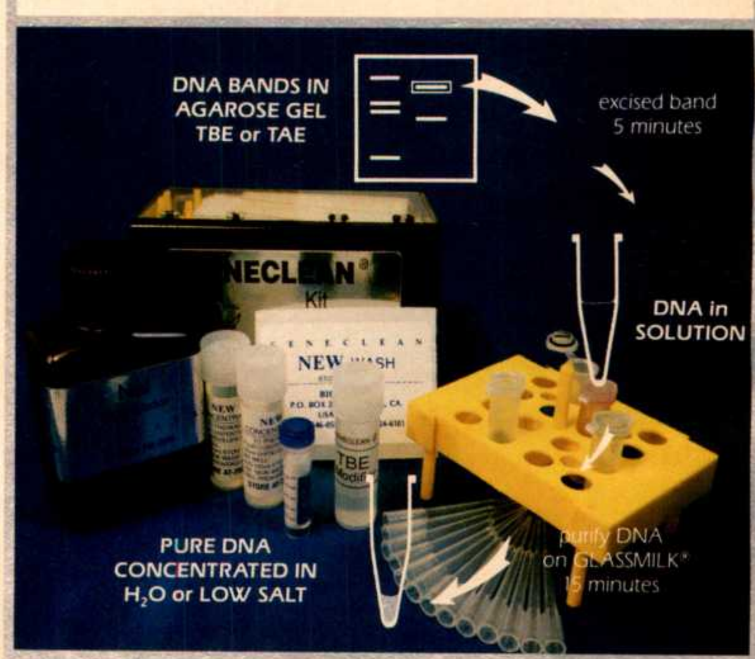
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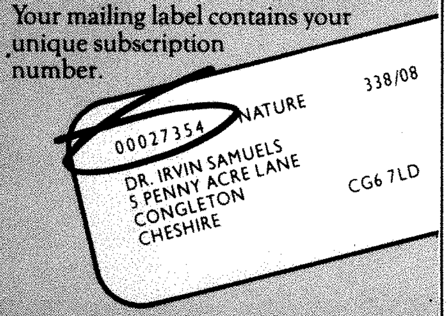

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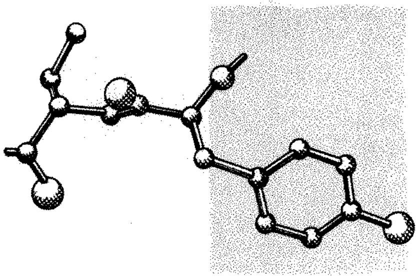

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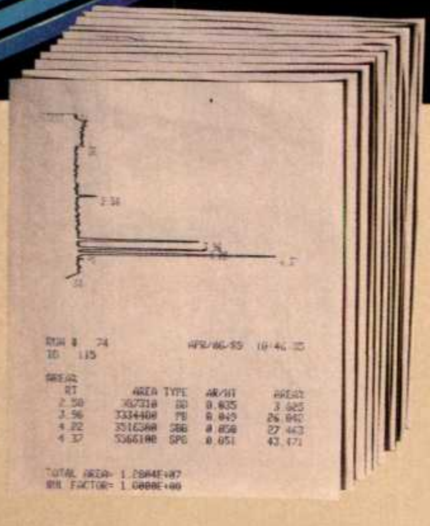

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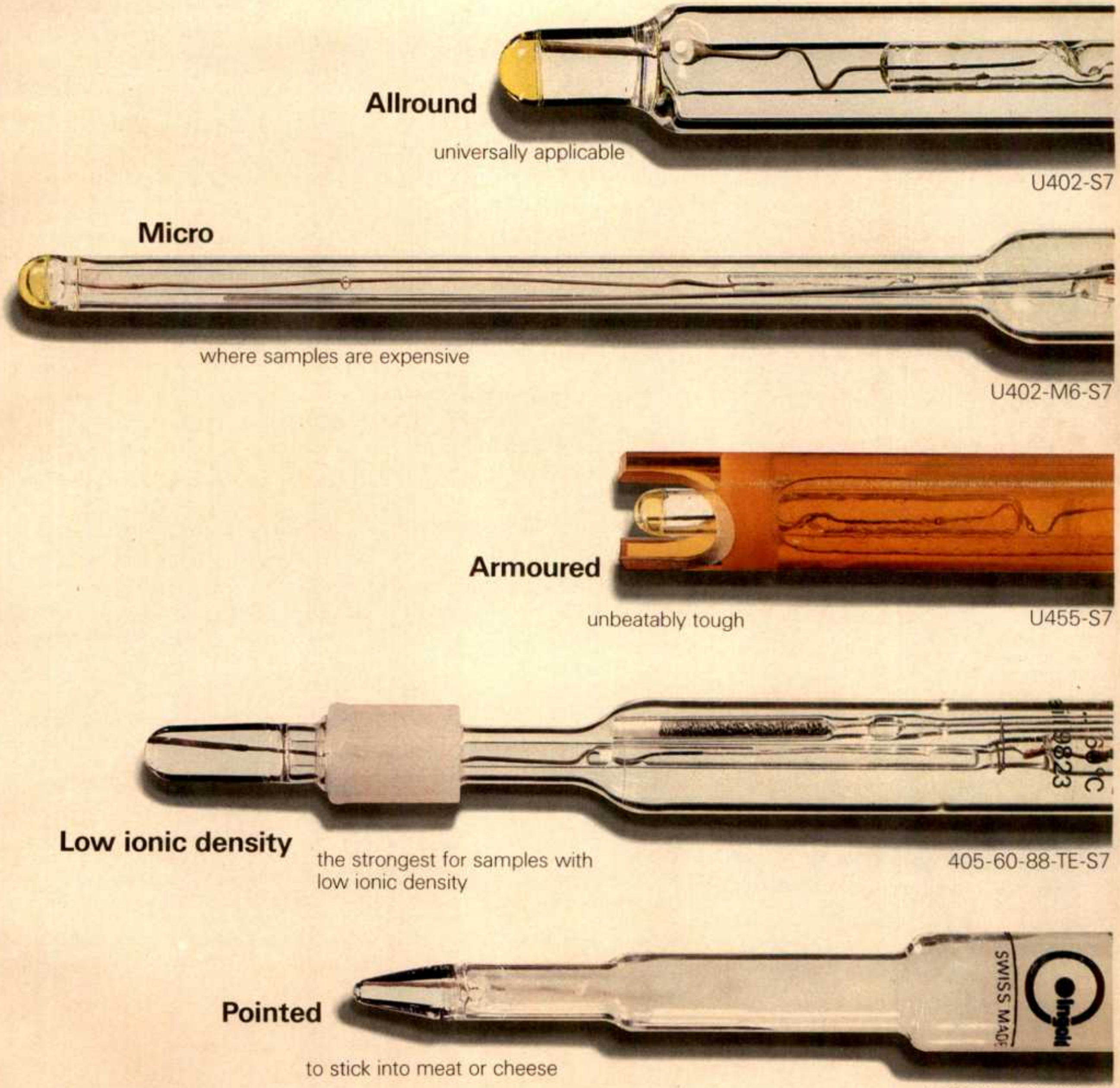

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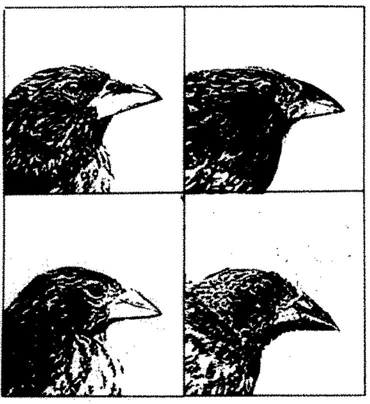

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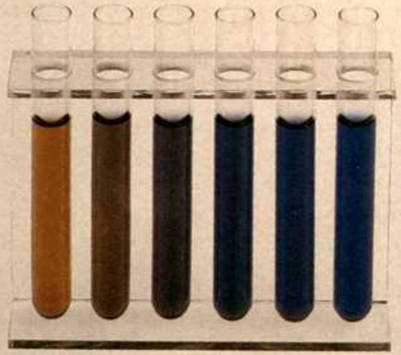

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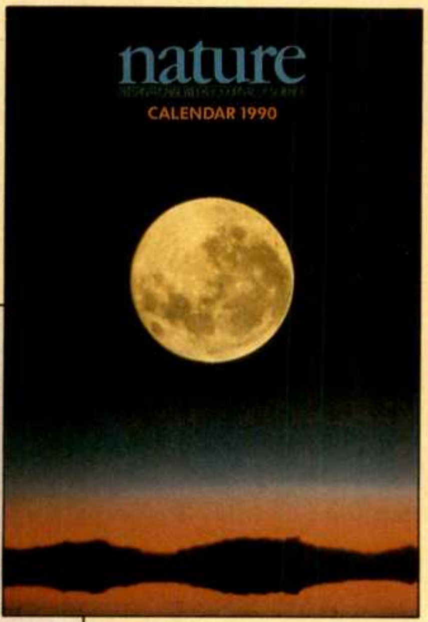

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Opportunity for Europe's science

One of the best, but smallest, of Europe's research organizations wants to be bigger. Not before time. But will those who hold the purse-strings agree?

NEXT week's General Assembly of the European Science Foundation (ESF) at Strasbourg could be crucial for the future of basic science in Europe. ESF's members, more than 50 research councils from 19 countries (with Iceland joining next week), will there have to take a view of the scale on which ESF functions. Huge sums are not at stake. The annual budget of ESF now is less than FF14 million, or less than $2 million. For the time being, the expansionists' plans would merely increase the scale of operations threefold. The choice is between the present shoestring and one only a little thicker. But there are two reasons why the issue is more important than that. First, ESF's budget is highly geared: each franc spent may be matched by 10 francs spent by member organizations. Second, the new activities on which ESF wishes to embark would give it, for the first time, an executive (if still small) role in the conduct of European collaboration in science. It thoroughly deserves one.

Here is why. ESF is catholic in the best sense. It follows the European mainland's broad understanding of what science is. The humanities and social sciences stand alongside nuclear physics and the exploration of interplanetary space. But ESF is deliberately not restricted geographically to the European Economic Community (EEC), but covers Scandinavia, Switzerland and Austria and even Turkey. People from Eastern Europe (and even China) have taken part as individuals in particular programmes. ESF now claims, rightly, that a nongovernmental organization such as itself is well placed to bring about more durable collaboration. Indeed, ESF seems to have been talking informally to two East European countries, but says that no "single dramatic initiative" is in prospect. ESF is certainly better placed to make a research bridge with the East then any other European institution. Will the tumultuous events of the past few weeks persuade next week's assembly to throw caution to the winds?

Success

As a club for European research councils, ESF has 16 successful years to boast about. It is organized to identify potentially fruitful fields for research collaboration, which are then carried through by groups of individual researchers supported, when necessary, by the research council members. The European Geotraverse Project, a coordinated programme (now almost finished) of deep seismic soundings from north to south in Europe (with a projection into North Africa) is a good illustration. ESF launched the project in 1983 at the urging of interested geophysicists, since when research councils electing to participate have paid for the direct costs of coordination (more than FF1 million this year) and have spent much more on research grants to support the actual work. The general opinion is that the implications of the data gathered will be more valuable than would have been the sum of the several uncoordinated parts. Much the same can be expected of the programme, likely to be approved next week, on the genetics of psychiatric illness, recent disappointments notwithstanding (see page 222). The plan is to harness resources on a European scale in the search for families likely to be useful in the search for genes implicated in psychiatric illness.

ESRS

But ESF has other feathers in its cap, most conspicuously the European Synchrotron Radiation Source now being built at Grenoble. The case for that was first made by an ESF committee. The funds were prised out of reluctant governments (chiefly West Germany and France) by ESF diplomacy. It is absurd that so little credit should have stuck to ESF for its midwife's role. This project in particular would have been carried through in half the time (seven rather than 15 years) if ESF had had more executive responsibility. ESF now says it wishes to play a more direct part in projects such as these. It could do worse than cut these new sharper teeth in the European VLBI Consortium's plan for the more effective coordination of data gathered by Europe's radiotelescope, still orphaned for lack of funds (see *Nature* **337**, 301; 1989).

What else is there to do? In the past few years, ESF has encouraged several successful informal networks of laboratories and individuals in fields as different as polar science and the use of longitudinal studies in childhood development. Again the principle is that ESF uses its own money to tell whether proposed networks will meet a need, often by organizing a sample workshop or two, whereupon administrative and operating costs are met by the participants. Now ESF wishes to go further — first by using its own money to organize research conferences and then by administering a scheme for up to 500 three-year postdoctoral fellowships a year. In a sharp break with previous practice, ESF would like to spend its own funds on these ventures, which means a bigger budget. Explicitly, ESF is saying that it has won the right to be regarded as the natural executive agent of its members for transnational collaboration on a European scale.

The argument is sound, but will it be heeded? Two issues are bound to dominate next week's debate: how promising are the new schemes, and can they be afforded? The underlying objectives are similar — to find ways of giving Europe's scattered academic communities a sense of being part of a critical mass. ESF hopes to be given funds next week to run a two-year pilot programme of conferences, but the case for the potentially more expensive postdoctoral programme is even stronger, if only because there is at present no general means by which people embarking on careers as independent researchers can be helped to move from one European academic centre to another. The underlying objective is to create within some European geographical context a sense that the academic community is a well-integrated whole, untrammelled by national or state boundaries. Until that happens, European science will not make the best of its potential and will remain at a disadvantage with North America.

But there are also difficulties. In breaking this new ground, ESF will, for example, for the first time encounter the too-familiar corrosive internal arguments about 'fair shares' that plague most international organizations: why should members from one country contribute when the immediate beneficiaries may be from elsewhere? The complaint mistakes the objectives, which are communal, not sectional. Specifically, postdoctoral fellows from one country who serve their time in a second and then become full-time academics in a third are only a 'loss' to the first two if the European academic community remains as balkanized as it is at present, which amounts to saying that neither ESF's plans nor other initiatives in the air will have much effect. That is a counsel of despair.

There are also practical difficulties; most postdoctoral fellowships are linked with research grants, for example, especially in the natural sciences. A postdoctoral fellow cannot work effectively without research funds, but universities are at present able to provide for only a few out of their own resources. But it would defeat the purpose of the new schemes if they were confined to fields in which research is relatively cheap, as in the social sciences. Uncertainties such as these, rather than the sheer cost of the postdoctoral scheme (which nevertheless could easily exceed the equivalent of $30 million a year) will determine what happens next week. It is to be hoped that ESF's assembly will be ready at least to experiment.

What matters most of all is that ESF's members should have a clear idea of why they are in business at Strasbourg. ESF is by no means the only European institution for the encouragement of transnational science, but it is a distinctive one. It differs from the organs of the European Economic Community (EEC), for example, geographically and by its concern for basic rather than applied science. (The EEC should pay more attention to basic science, but that is another matter.) ESF is also concerned with quality in research, not merely with getting things done. However much it grows, it will not in the foreseeable future be a big spender, but its record shows that its influence can far exceed the scale on which money runs through its hands. The opportunity, now, is to recreate the circumstances of the eighteenth century, when European scholarship was free and also European in the broadest sense — manageably so because it was so much smaller. The prize, the reinvigoration of European scholarship — could also be disproportionately handsome. □

Strength via adversity?

Having outlasted an administrative attack, the US fusion programme must not subside into somnolence.

THE only thing worse than a federal science administrator who knows nothing about science is one who does. That, apparently, is the lesson that physicists in the US magnetic fusion programme will be obliged to draw from the recent departure of Robert Hunter from the Department of Energy, where he had been director of energy research. In Congress and elsewhere, those who have been trying for decades to persuade tokamaks to burn deuterium and tritium had been complaining about Hunter's activities: he diverted funds from magnetic to laser fusion research, tried to stall construction of the Compact Ignition Tokamak and, worst of all, refused to take physicists at their word when they assured him the magnetic fusion programme was proceeding as planned towards commercial energy generation.

Hunter was, no doubt, politically a clumsy man. He shifted money from one budget category to another without consulting the appropriate Congressional committees. He further irked Robert Roe, the chairman of the House Committee on Science, Science and Technology, by observing in a departmental memorandum that Princeton was not the automatic choice for the site of a new tokamak because of political concerns over radioactivity; Roe, coming across this note at a fractious hearing, was in no mood to be told by a federal administrator what was or was not a political issue in his home state.

There will be many sighs of relief and little lamentation at Hunter's going. But before the tokamak community congratulates itself too much, they should ponder something: for the first time since the oil crisis of the early seventies, fusion energy was the subject of a noisy political debate. A dozen congressmen grappled intently with plasma quality factors and confinement times. In fighting off Hunter's attack, scientists who had languished under level funding for fifteen years found themselves being praised by some influential voices. With a more timid and less engaging character in Hunter's place, the magnetic fusion programme may well return to the political backwaters. Physicists had better make sure that this does not also mean a return to the financial neglect and sloth of the last decade. □

Nuclear installations not the cause of cancer?

- Risks similar at rejected and chosen sites
- Unidentified risk factor may be involved

Washington

RESEARCH published in Britain last week shows that the mortality rate from leukaemia and Hodgkin's disease in young people is higher not only near existing UK nuclear power stations but also near places being considered as future power station sites. This further tips the balance of the evidence away from the theory that radioactive discharges are behind the increases.

Epidemiological research in Britain, the world leader in studies of the health effects of nuclear installations, has so far been inconclusive in attempts to prove or disprove a link between radioactive discharges and an increase in the incidence of leukaemia and Hodgkin's disease in young people living nearby.

After examining the evidence last year, the Committee on Medical Aspects of Radiation in the Environment, an independent commission, concluded that some unidentified feature of Britain's nuclear reprocessing plants at Sellafield in Cumbria and Dounreay in Scotland was the most likely cause of the increased incidence of leukaemia, but could not attribute the increase to radioactivity in the area because the measured levels were so low.

Leo Kinlen of the Cancer Research Campaign's epidemiology unit at the University of Edinburgh later found evidence to support the theory that the increased leukaemia incidences might result from viral infections. His theory proposes that a rapid influx of population in the vicinity of a nuclear installation, usually located in an isolated area, brings an infection to which the indigenous population has not evolved resistance.

In an attempt to decide whether the increased cancers are caused by radiation from nuclear installations or by some other contingent feature, Paula Cook-Mozaffari, Sarah Darby and Richard Doll of the Imperial Cancer Research Fund Laboratories in Oxford compared mortality data from areas near two Central Electricity Generation Board (CEGB) nuclear power stations and six other sites being considered by the CEGB for the construction of power stations. They reported in *The Lancet* last week that the increased risk of mortality due to leukaemia and Hodgkin's disease in young people was "strikingly similar" in both groups.

Although they declined to suggest any reason for the increased mortality, they suggested that these areas might share "important unrecognised risk factors" other than environmental radiation pollution. But they also point out that there had been no sudden population influx in the potential sites, which argued against the viral infection theory.

Evidence is now mounting, says Darby, against the theory that radioactive discharges are responsible for the increased cancer risk, although at Sellafield, home to one of Britain's two nuclear reprocessing plants, the discharges and the increased cancer risk are much higher and the issue is still open. At Dounreay in Scotland, the site of Britain's other reprocessing plant, the reported increased leukaemia incidence is also significantly higher than in the areas in this study.

Kinlen says the new results are consistent with the theory that in isolated coastal areas, which would be suitable sites for nuclear installations, the pattern of human interaction is different from that in the average large inland town and could explain the increased risk for certain types of cancer. He adds that "it is possible, if not probable" that the new results are recording the same effect he saw on a smaller scale and predicts that a similar result would be found in any rural coastal area, regardless of whether it was a potential site for a nuclear power station.

Christine McGourty

Cancer near potential sites of nuclear installations, by P. Cook-Mozaffari, S. Darby & R. Doll, *Lancet* **II**, 1145–1147 (1989).

NUCLEAR POWER

Privatization spells end of a nuclear dream

Washington

THE future of the nuclear industry in Britain is in doubt after last week's announcement by the government that it was abandoning plans to build three new PWRs (pressurized water reactors). The reversal of the plans to expand the nuclear industry is an about-face by the Conservative government under Prime Minister Margaret Thatcher, a staunch advocate of nuclear power, but was inevitable given the government's determination to privatize the electricity industry and the refusal of the soon-to-be-privatized local electricity boards to carry through the £7,000 million PWR programme and face the huge costs of nuclear waste disposal and of de-commissioning old power stations.

Strong opposition to nuclear power forced a long and controversial public inquiry into the safety and design of Britain's first PWR at Sizewell in Suffolk. The plans were approved 18 months ago and construction is under way. The subsequent inquiry into a second PWR, at Hinkley Point C in Somerset, has been running for more than six months and was was drawing to a close, but will now be abandoned along with plans for another two PWRs at Sizewell C, and Wylfa B in Wales.

Opponents of nuclear power scored a major victory during this inquiry when the Central Electricity Generating Board, which has always promoted the low costs of nuclear power, was forced to disclose figures showing that its coal-fired power stations are cheaper.

In an attempt to ensure that nuclear power was not neglected after privatization, the government had stipulated that by the year 2000, 20 per cent of the electricity demand must be met by non-fossil fuel sources to maintain fuel diversity. A new private company, National Power, was to be responsible for nuclear power generation. But its chairman-designate, Lord Marshall, is confidently expected to resign; and now the 10 existing Magnox reactors and 7 AGRs (advanced gas-cooled reactors) will be retained in a new publicly owned company.

Former director of the UK Atomic Energy Authority, Lord Marshall may not be the man to run a coal-fired CEGB.

Christine McGourty

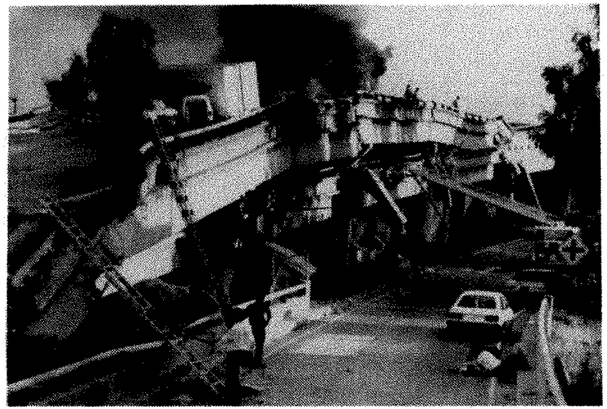
LOMA PRIETA EARTHQUAKE

Testing time for Caltrans

San Francisco

A SIMULATED earthquake on a portion of intact freeway and an elaborate computer model of the San Francisco Bay Bridge are among the studies likely to be adopted by California officials in an effort to understand what happened during the 17 October Loma Prieta earthquake. At a meeting scheduled for late last week between representatives from Caltrans, the state transportation department, and University of California engineers, several proposals for roadway studies were due to be debated, but Caltrans spokesman Jim Drag confirmed that the simulated quake and the Bay Bridge studies seemed likely to be approved.

Also last week, California governor George Deukmejian signed 24 earthquake relief measures, including a sales tax increase of a quarter-cent per dollar, and officials announced that the Bay Bridge would reopen on 17 November, exactly one month after the quake.



Looking south on Interstate 880 on the Tuesday evening. (AP).

The highway test involves an intact section of Interstate 880 in Oakland, just south of the Cypress Viaduct section which collapsed during the upheaval. Apart from a firmer foundation, the section is almost identical to the portion that collapsed. The prevailing theory is that the upper deck failed because the supporting columns and the joints that bind them with the lower deck had not been adequately reinforced. Two tests will examine these and other ideas.

In one, hydraulic jacks mounted on steel frames under both sides of the roadway will be used to simulate the sideways movement of the 7.1-magnitude quake. An array of electronic instruments and cameras will measure the displacement and strain developed at specific points, and record the physical damage.

The overpass will first be tested in its present condition, after which engineers will reinforce the columns with steel beams, and then slowly bring the freeway to the failing point, which might take as much as three million pounds of force. The objective, says Jack Moehle, a structural engineer at the University of California at Berkeley, is to test proposed strengthening techniques for other San Francisco Bay Area double-decker freeways. Three similar roadways in San Francisco that were damaged in the earthquake are still closed.

In the second test, which will alternate with the hydraulic-jack experiment, a pair of vibration generators will be attached to the same freeway section. Attached to the upper deck, the twin 'shakers' rotate heavy weights in opposite directions at a synchronized frequency, so that the centrifugal force is focused in a specific direction along the roadway. The force, of the order of 10,000 pounds, will not be great, but by repeated application at a steady rate it will test the structure's endurance against vibration.

Accelerators attached to the freeway will allow engineers, knowing the mass of the deck, to measure the forces developed in the supporting columns. "If we know the total force in the structure and how it displaces under that force, we can develop a good computer model of the resisting mechanism", says Moehle.

The tests will be finished by Christmas, when the entire freeway span will be demolished. Because the tested section will not be "failed" in its original condition, engineers will have no direct measure of how the Cypress section really collapsed, but the investigators hope that demolition crews will haul a few columns away for further study in the laboratory.

The Bay Bridge study will be confined mainly to laboratory simulations and computer modelling. Faculty and students from the structural engineering programme of UC Berkeley's civil engineering department have alredy been amassing damage information, from notebooks, photographs, videotapes and sketches. They plan to supplement this data with other input to develop a detailed computer model of the bridge that extends even to its underlying soil.

"Our main goal is to make sure our computer programs — simulations, modeling, all the tools that we have to do the analysis — are capable of predicting events like this and damage like this", said Abolhassan Astaneh, a UC Berkeley assistant professor of civil engineering. These studies, he hoped, will enable engineers to recommend specific retrofitting plans and other modifications, so that the bridge can withstand "the big one".

Robert Buderi

COMPUTER SOFTWARE

Federal policy makes trouble

Washington

MANY examples of "waste, fraud and abuse" in the federal government can be attributed to problems in developing software, according to a report released last week by the US House of Representatives Committee on Science, Space and Technology. On everything from budgeting to intellectual property rights, it says, government policies "have congealed over time in a manner almost perfectly designed to thwart the development of high-quality software".

Although no single action will instantly solve the government's software woes, the committee staff recommends that a working group on improving software development be set up in the president's Office of Science and Technology Policy with the aim of making long-term changes in the government procurement system.

At present, government contracts specify in "excruciating" detail exactly how a system will look at delivery several years in the future, so when the inevitable changes are introduced, software quality is adversely affected and development costs increase.

Reducing overall costs would require spending more time and money in the design stage, when it is much cheaper to correct an error, than in the maintenance phase. But this strategy is discouraged by the process for obtaining congressional approval, which requires initial costs to be minimized.

The report, stemming from an investigation into faulty software in a radiation therapy machine which caused several deaths, also calls on the National Institute of Standards and Technology to play a greater role in improving software evaluation. At present there is no infallible method for assessing quantitatively the safety of software used in, for example, aircraft flight controls or nuclear reactors, in which software flaws can put lives at risk. The federal government's "software woes" are exacerbated by the staff crisis created by its inability to compete with the private sector in attracting software specialists.

Computer security and ethics also came under scrutiny last week in the House subcommittee of criminal justice, which is considering two bills to increase the penalties for damaging hardware and software as a deterrent to hackers. But researchers and legal experts are divided over the need for new legislation, definitions of "authorized access" and how to increase computer security without restricting free exchange of information. Action on the bills is being delayed pending the outcome of the trial of Robert Morris, the Cornell University graduate student whose 'virus' disabled thousands of computers last year.

Christine McGourty

COLD FUSION

Official thumbs down

Washington

To no one's surprise, the panel set up by the Department of Energy (DoE) to assess cold fusion has sent in a negative report. An interim opinion released earlier this year by the panel, a group of 23 electrochemists, nuclear physicists and materials scientists assembled by the DoE's Energy Research Advisory Board (ERAB), acknowledged that there were some odd and unexplained results in the air but recommended against any special support of cold fusion experiments (see *Nature* **340**, 174; 1989). The panel's final report expresses the same verdict a little more forcefully, but the ERAB meeting at which the report was approved and passed on to DoE Secretary James Watkins did reveal some differences of opinion on the significance and credibility of the handful of 'positive' results still extant.

Summarizing the final report, John Huizenga, a professor of physics and chemistry at the University of Rochester and one of the ERAB panel's co-chairmen, showed little patience with those who have continued to claim that there is at least a little evidence for cold fusion of the heat-producing variety. None of the calorimetry, he declared, was of good enough quality to be believed, and in any case calorimetry alone would never prove the case. Huizenga was particularly unhappy with those who argued that because the claimed excess heat could not possibly be of conventional origin it had to be nuclear, despite measurements which showed unequivocally that no fusion products were being generated.

Discounting the calorimetry, the only anomalous claim still needing an explanation was that of Kevin Wolf of Texas A&M University, who repeatedly finds tritium in his electrochemical cells. But new evidence from Wolf, discussed at last week's ERAB meeting, argues against a nuclear origin. Tritium produced by deuterium fusion comes with an energy of 1 MeV and is accompanied by a 3 MeV proton. Energetic tritons will react with deuterium, either in the palladium electrode or in the electrolyte, producing secondary neutrons, and the protons can excite some palladium nuclei, which give up a 500 keV photon on de-excitation. In his latest experiments, Wolf has searched for both these secondary signatures and found nothing. His tritium, therefore, must have some kind of low-energy origin.

Nevertheless, Wolf has also searched minutely for sources of contamination, and found nothing there either.

This puzzling result, together with a few calorimetric measurements that he found somewhat convincing, led recent Nobel prizewinner Norman Ramsey of Harvard University (see *Nature* **341**, 556; 1989), the panel's other co-chairman, to conclude that the case against cold fusion was not closed. At his insistence, a preamble was added to the report's conclusions which notes that "it is not possible to state categorically that all the claims for cold fusion have been convincingly either proved or disproved".

Huizenga felt that this preamble "weakened" the report, but Ramsey and some others felt that the report would undermine itself if it looked too dogmatic in its dismissal of the evidence.

On the subject of the low-level fusion process espoused by Steven Jones of Brigham Young University, the panel agreed that more work was needed, although here again Huizenga, noting that all the claims were of marginal statistical significance, seemed less impressed than some of the other panel members that there really was a phenomenon to be explained.

Although he showed some sympathy for those still making claims for cold fusion, Ramsey also said that if the results on which the claims were based came from his laboratory, he would keep quiet and do more experiments until he understood better what was going on. The ERAB report accepts that people are free to submit experimental proposals to granting agencies in the hope of obtaining support for cold fusion experiments, but recommends that only if experiments are likely to yield useful results — closed-cell calorimetry with simultaneous nuclear monitoring is deemed essential — should support be given. **David Lindley**

EAST GERMAN EXODUS

Problems remain for those who stay

Munich

EAST German researchers now living in West Germany are happy but sceptical about the events in East Germany that culminated in last week's opening of the Berlin Wall. No one disputes that the opening of the borders is a momentous and irreversible step, but many doubt that the effects of 40 years of Communist repression can be wiped away overnight: the repressive system is more deeply entrenched in East Germany than, for example, in Hungary, which has also enacted radical reforms this year.

Under the old system, said a number of researchers, Party members and other desirables were given the opportunity to travel abroad, publish in foreign journals and make progress in their scientific careers, but those who lacked an ideological pedigree were cut off from the West and forced to engage in political activities against their will. The pessimism brought on by such obstructions caused these people, like so many others, to escape.

Those who tried to leave by legal means found that as soon as they submitted visa applications, they were forbidden to work in their laboratories. One said he was reinstated only after an invitation to a scientific meeting from a prominent West German research institution, in which he now has a job. But the only way to find out about such opportunities is to have personal and professional contacts, said another.

Researchers said after the recent lifting of travel restrictions that they were waiting to see if the changes are large enough to encourage development of a system based on personal achievement and not privilege. Even so, East German research laboratories will still lack the money to order basic supplies, let alone to subscribe to foreign journals. Steven Dickman

West Germany continued to welcome all those who came over from East Germany on Friday, even though the capacity of the refugee camps had nearly been exhausted. Most of the refugees who had arrived before the Wall came down seemed to be in practical, not academic, professions, although an accurate tally was made impossible by the large numbers. But organizations involved in helping East German researchers and academics get on their feet in West Germany said they would need much more money to continue the process.

Uta Paffhausen of the Education Ministry reported that the number of applications for "adjustment aid", given to help East German emigrants find academic posts, had risen to about 50 in the past two months. The grants are provided by the ministry as well as by the Otto Benecke Foundation. The ministry also administers a programme to provide fellowships for refugees whose training is unsuitable for working in West Germany. The two programmes were to provide a total of DM 24 million ($13 million) in 1990 when the refugee total was still expected to be about 60,000. But by 10 November, the number had topped 500,000, including 200,000 East Germans and another 300,000 ethnic Germans from Poland and the Soviet Union. Photo: The wall near Checkpoint Charlie last Friday, seen from the East (AP).

GLOBAL CHANGE

Politicians still only talking

Boston

In a week marked by vocal debate over global climate change, a group of representatives from private foundations gathered quietly with scientists and international security experts in Cambridge, Massachusetts, last week to explore the international political fallout from anticipated changes in the environment.

While British Prime Minister Margaret Thatcher called upon the UN General Assembly to lead the way in worldwide action, and environmental policy-makers from 68 nations gathered in the Netherlands failed to agree on international limits on carbon-dioxide emissions, those meeting in Cambridge tackled social and legal issues such as the prospect that drastic climate change could produce 'environmental refugees' and the idea that environmental concerns could become so crucial that countries would be justified in overriding the sovereignty of others to enforce protective measures.

At the Cambridge meeting, which was sponsored by the American Academy of Arts and Sciences, the presence of representatives of some of the largest private US foundations signalled a mounting interest in global change research. Although no new financial commitments were announced at the meeting, several foundations, including the John D. and Catherine T. MacArthur Foundation and the Social Science Research Council, have already earmarked funds specifically for research on global environmental change.

But dissent over political choices overshadowed scientific uncertainty. The scientists present agreed that precise and consistent models of global climate change are decades away, but nonetheless presented a fairly uniform and troubling consensus about the threats posed by ozone depletion and trapped greenhouse gases. It was the social scientists and security experts who clashed most severely in their assessment of the political implications of the issue.

Some, like George Rathjens, professor at the Center for International Studies at the Massachusetts Institute of Technology, argued that the economic costs involved in curbing emissions may be too high to allow immediate action, and that the costs "diminish the prospects for international agreements". But others declared that "we know enough now" to justify a push for international cooperative measures — even coercive measures.

Speaking unofficially on behalf of the Brazilian government, Eunice Durham, an anthropologist at the University of São Paulo, bristled at even the notion of international coercion or pressure to reduce global warming. In the view of the Brazilian government, said Durham, "military action [would be] justified" if the nation's sovereignty over the Amazon rain forest is in any way challenged. Furthermore, she added, deforestation "is not going to be stopped unless there is a viable alternative development plan for the region". Similar divisions and disagreements emerged at the international conference in the Netherlands. Despite President George Bush's claims of the need for a "war against global warming", the United States, together with Japan and the Soviet Union, led the way in thwarting the endorsement of a specific timetable for curbs on emissions of carbon dioxide. Most of the 68 countries present at the Netherlands environmental conference wanted to freeze allowable emissions of carbon dioxide by the year 2000.

William K. Reilly, head of the US Environmental Protection Agency, stressed the fact that the United States agreed in principle to the need for international emission levels. But many critics in the United States and elsewhere were disappointed at the outcome of the Netherlands meeting, and criticized the United States for its deliberate inaction. As one critic put it, the US position made Bush's strong statements about global warming look like "a lot of hot air". **Seth Shulman**

GLOBAL KNOWLEDGE

Americans and Russians mostly at sea

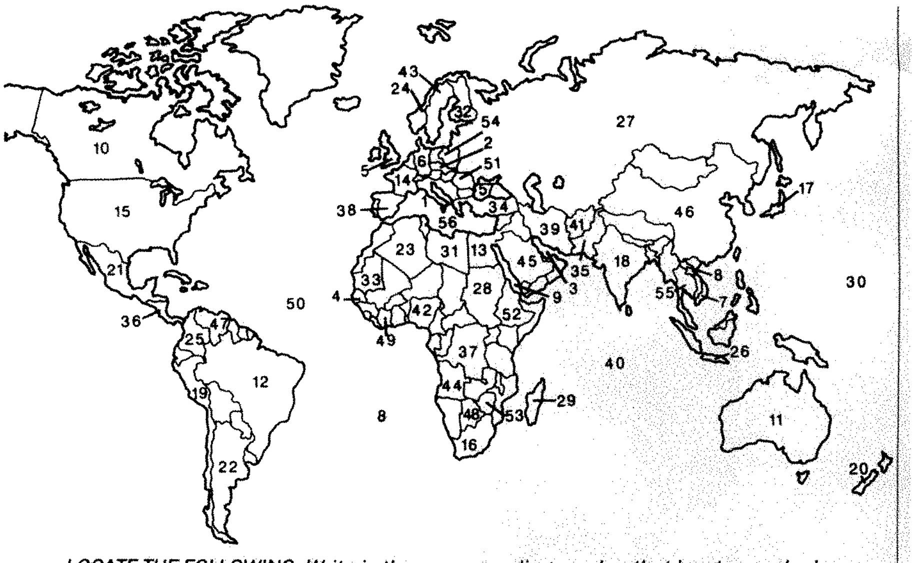
Washington

When George Bush and Mikhail Gorbachev meet upon the Mediterranean Sea next month, they will be on equal terms in at least one respect: their respective countrymen will have an equally vague idea of where in the world they are. Asked to identify 16 locations on the map below, US citizens got an average of 8.6 correct, and Soviets did a little worse, scoring 7.4. Swedes and West Germans did best (11.6 and 11.2 correct identifications); the Japanese averaged 9.7 and the British 8.5.

The results of the survey, conducted by the Gallup organization for the National Geographic Society specifically to compare knowledge of geography in ten countries, have been taken in the United States as more evidence that the educational system, no matter how many brilliant scholars it may produce, is failing the general population. For Americans, the most alarming result of the country-identification quiz was that the youngest age-group (18–24) did significantly worse than the rest of the population: for the other countries, younger people tended to do as well as or better than their elders. Last year, an international survey put US high school students near the bottom of the class in science (see *Nature* 332, 195; 1988).

But the Soviets, if the responses of 1,500 inhabitants of Moscow and Kursk reflect the knowledge of the population as a whole, have even more reason for alarm. Although 88 per cent of the Soviet sample had taken a geography course in school, and 61 per cent thought geographical knowledge a necessity, their achievements belied these ideals. Only 38 per cent of the Soviets knew where Afghanistan was.

David Lindley



LOCATE THE FOLLOWING: Write in the corresponding number that locates each place. Answers on page 218. (Based on NGS questionnaire.)

— *United States*
— *Soviet Union*
— *Central America*
— *Japan*
— *Canada*
— *France*
— *Persian Gulf*
— *Mexico*
— *Italy*
— *Sweden*
— *United Kingdom*
— *South Africa*
— *West Germany*
— *Pacific Ocean*
— *Egypt*
— *Vietnam*

AGENT ORANGE

'Not guilty' verdict challenged

Sydney

FOUR years after a Royal Commission adjudged that the widely used defoliant Agent Orange had caused no measurable or lasting ill-effects in soldiers in Vietnam, a group of Australian scientists has called the commission's findings unscientific. Eighteen biologists, chemists and immunologists from some of Australia's leading universities held a conference in Canberra last April under the title *Evatt Revisited,* in reference to the 1983 Royal Commission on the Use and Effects of Chemical Agents on Australian Personnel in Vietnam, headed by Justice Phillip Evatt. The proceedings of the conference were published last week, and include the charge that the commissioners simply duplicated large sections of the submission by Monsanto, the US chemical company that makes Agent Orange.

The Evatt Royal Commission was set up to determine whether Australian servicemen who served in Vietnam were more likely than others to suffer from cancer and whether their children had a higher probability of birth deformities. Since the commission released its findings in July 1985, it has been the target of complaints from the Vietnam Veterans Association of Australia (VVAA), from witnesses who testified before it and now from some of Australia's most senior scientists. Critics say, among other things, that the report is shallow and extravagantly worded, that scientific evidence was poorly interpreted and that VVAA witnesses were treated as "hostile".

In 1987, the federal government ordered an inquiry into the findings of the Royal Commission, and concluded that the commission should not have come out with its "not guilty" assertion. The report maintained that the commission's findings and conclusions were sustainable but that it lacked credibility because of the manner in which the inquiry was conducted. But the new criticisms raised in *Evatt Revisited* are the first to complain about the commission's handling of the scientific evidence.

Ted Steele, a biologist from the University of Wollongong and one of the editors of *Evatt Revisited,* says that the commission's conclusions that exposure of civilians to Agent Orange had little or no effect on birth abnormalities was not warranted. He says that new evidence of birth abnormalities has come forward since the Royal Commission did its work, and believes that such findings "suggest that more cautious recommendations should have been forthcoming both from the Royal Commission and their scientific advisors". But Steele also has a more serious charge: he says that "large tracts of the report were plagiarized verbatim from the submission of Monsanto". But John Coombs QC, counsel assisting the commission, says Steele's allegation is "nonsense". He points out that it is common practice for a commission to incorporate appropriate parts of the submissions received into its report, and adds that "part of the VVAA submission was also reproduced".

Tania Ewing

TOXIC WASTE DISPOSAL

Commercial plans delayed

Cape Town

PLANS by a Cape Town company, Peacock Bay Environment Services, to build a toxic-waste disposal plant that would process imported as well as domestic waste have been put on hold by Gert Kotze, South Africa's minister of the environment. Kotze announced last month that the government would not consider licensing the importation of toxic waste before it had been presented with the results of a newly commissioned survey on domestic waste disposal. The survey will be conducted by the CSIR's Foundation for Research Development.

According to Sidney Sanders, a director of the company, the importation of foreign waste is essential for the economic success of the proposed R400 million ($160 million) development.

He also claims that the plant offers "the first viable alternative to the inadequate way in which toxins are being disposed of in this country". But unease over the plans derives more from worries over the transportation of waste than its treatment at the plant. A local toxic-waste transport company has indicated its reluctance to transport more waste by road, because the risk of accidents is too high, but Peacock Bay proposes to use the rail system instead.

The development, whose intended site is Alexander Bay, on the west coast 600 km north of Cape Town, is believed to be the first proposed under the new Environmental Conservation Act, promulgated by parliament in June of this year. The Act makes environmental impact assessment a legal prerequisite, and the Peacock Bay company says it has followed the procedures laid down by the act "to the letter", and has spent R750,000 ($300,000) on an impact assessment study.

But local environment groups, spearheaded by the newly formed pressure group Earthlife Africa, have come out against the idea of importing toxic waste. Kotze, meanwhile, has accused Earthlife Africa of sowing suspicion about the government's integrity, but his department nonetheless appears to be sensitive to public reaction.

Michael Cherry

US SECURITY

FBI interference with libraries criticized

Washington

FRESH concerns arose last week over interference by the Federal Bureau of Investigation (FBI) in the operation of academic and other public libraries. A year ago, the FBI came under attack for its long-standing Library Awareness Program (LAP), in which it tried to enlist the help of librarians in identifying foreign spies who might be seeking access to scientific and technical libraries (see *Nature* 336, 507; 1988). Now documents obtained by the National Security Archive (NSA), a non-profit Washington library and research centre, reveal that librarians who refused to help the FBI or publicly criticized LAP were subsequently investigated to see if the campaign against LAP was itself part of a Soviet plot.

The NSA, whose collection includes a variety of unclassified and declassified government materials, requested documents concerning LAP under the Freedom of Information Act, and then filed a follow-up lawsuit when the FBI failed to deliver. Under an agreement reached earlier this year, the FBI has now released 1,200 pages of internal documentation, blacked out in some places.

The FBI memoranda reveal that activities under LAP were continued through the past two years despite assurances from senior officials to the American Library Association (ALA) that the programme was put in abeyance after December 1987. FBI agents were, however, told not to refer to LAP by name because of the adverse publicity. And one internal FBI communication, dated 6 February 1989, notes that although 266 individuals had been subjected to "indices checks" since November 1987, only eight "references" were found. The purpose of these background checks, according to the FBI memo, was to "determine if a Soviet active measures campaign had been initiated to discredit the Library Awareness Program". The nature of the eight "references" turned up is not revealed.

But according to an FBI spokesman the ALA and the NSA are reading sinister motives into routine matters. Whenever anyone writes to the FBI on any subject, files are checked to see if the person has had any previous contact with the FBI, has been investigated by the bureau, has a criminal record, and so on. The "indices checks" referred to in the memo are simply searches through existing FBI files to see if a positive entry exists in any one of about 250 categories. The spokesman emphasized that these checks do not involve sending out agents to conduct personal investigations or questioning. Nevertheless, lawyers for the ALA are sifting through the 1,200 pages of FBI documents to see if there are any grounds for further action.

David Lindley

GENETIC ENGINEERING

New law is overdue

Munich

A West German court last week ordered Hoechst AG to cease construction of a nearly completed plant that would use genetically engineered bacteria to make human insulin. The court ruled that it could not approve the licence granted for the construction of the plant because there is no legal basis for regulating the use of genetic engineering. The decision increases the already intense pressure on the government to pass a law regulating genetic engineering in factories and laboratories.

The court rejected the relevance of the West German Federal Emission Protection Act, which had been used as a legal basis for constructing at least one other facility using genetic engineering. The decision reversed earlier rulings by lower courts which had upheld Hoechst's right to build the plant despite legal challenges from local residents. Hoechst may appeal to the federal constitutional court at Karlsruhe for a reversal of the decision on constitutional grounds, but has not yet decided if it will do so.

The Hoechst plant would use the bacteria *Escherichia coli* to produce human insulin, as US companies have been doing for a decade. Hoechst has invested an estimated $60 million in the plant and a spokeswoman said that the company intends to "fight further" for the use of genetic engineering in drug manufacture in West Germany. She called it "astounding" that there could be so much fuss over a technique that uses *E. coli*, which is found in the human intestine.

But the debate over genetic engineering here has been marked by the almost religious belief held by opponents that all genetic engineering is hazardous. Although declining to rule on ethical or safety aspects, the court seemed to agree, saying that genetic engineering represented a "new dimension and quality" of technology with "risks for human beings and the environment that could not be properly assessed at this time . . . . Only the democratically elected legislature" can decide how genetic engineering could be regulated.

The court's decision adds urgency to efforts to speed passage of a 'basic law' for genetic engineering that has been under discussion all year (see *Nature* **340**, 85; 1989). The cabinet published its views on the latest version of the law on 9 November, the day after the court decision, preparing the way for discussion in the Bundestag this week.

It made some concessions to the *Länder* (states), for example agreeing to more public participation in the licensing procedure, but rejected the request of the *Länder* that they be allowed to carry out the provisions of the new law. The cabinet warned that this could potentially lead to a variety of interpretations of the law, which would be bad both for industry and for basic research.

But before the law can be put into practice, detailed regulations must be drawn up by the Health Ministry and the regulations attached to the law are "as important as the law itself" for basic research, said Ernst-Ludwig Winnacker, vice-president of the Deutsche Forschungsgemeinschaft (DFG). The effect on basic research "will depend on the wording", he said, and DFG, which is the main source of outside funding for basic research at universities, has volunteered to help draw them up.

Steven Dickman

ANTI-CLOTTING DRUGS

Approval for TPA competitor

Washington

The approval of SmithKline Beecham's blood-clot-dissolving drug Eminase by the blood and blood products committee of the Food and Drug Administration (FDA) on 31 October puts the drug just one step away from being released for sale on the US market. Eminase, a complex of the already available thrombolytic drug streptokinase with human plasminogen, had been expected to win approval several months ago, but was held up for additional scrutiny by an FDA advisory committee amid reports of pressure from Genentech, which manufactures the rival drug Activase (tissue plasminogen activator, TPA).

David Stump, Genentech's director of clinical research, reported to the advisory committee that he had evidence of antibody production in response to the plasminogen portion of Eminase.

But the results presented by SmithKline Beecham indicated no evidence of increased autoimmunity. Alan Wachter, manager of scientific information at SmithKline Beecham, emphasized that the approval of Eminase was unanimous. Although no price has yet been set, the company says the drug will be cheaper than existing thrombolytic drugs. Eminase has a longer half-life in the bloodstream than streptokinase, and can therefore be administered in 2–5 minutes instead of being infused over an hour. Activase has to be given over a period of three hours.

A large mortality study comparing the efficacy of Eminase, streptokinase and Duteplase (Boroughs Wellcome's TPA) is in progress and results are expected within the next few years. **Diane Gershon**

IN VITRO FERTILIZATION

Political pressure means poor research

Washington

In a report issued last week, the Institute of Medicine (IOM) *called on the NIH to* coordinate vigorous research programmes in basic reproductive biology and in the technologies of medically assisted conception in order to improve the practice of *in vitro* fertilization (IVF) and embryo transfer in the United States. Neglect of research in this politically sensitive area, the report says, is slowing the development of better methods of contraception, better techniques to preserve endangered species and more cost-effective methods of food production.

At present, embryo and fetal research require the approval of the government's Ethics Advisory Board (EAB). But the board has has not been reconstituted since its charter expired in 1980. The IOM report complains that the eight-year-old ban on the use of federal funds for this kind of research means that more is now known about reproduction in commercially valuable animals such as cattle than in humans. Moreover, the clinical practice of IVF and embryo transfer is outstripping its scientific foundations.

After the renewal two weeks ago of the ban on research involving the transplantation of fetal tissue into humans (see *Nature* 342, 105; 1989), there is little optimism that any steps will be taken to revive the EAB. The IOM recommends that if politics paralyses the existing review mechanisms, then a nongovernmental organization, along the lines of Britain's Voluntary Licensing Authority, should be established to develop guidelines for embryo and fetal research.

But in addition to the political pressures, the report says that the lack of vocal advocacy groups to fight for major increases in federal support, the lack of communication between researchers in the different fields of reproductive biology and the lack of research material for experiments in humans and other primates all contribute to the slow progress in this field and to the low success rates of IVF and embryo transfer.

To improve the safety and effectiveness of the techniques used, the IOM suggests that the professional societies should establish a mechanism to collect and monitor data from clinical centres.

Christine McGourty

World map answers

Answers to NGS map questions (page 216). United States (15), Soviet Union (27), Central America (36), Japan (17), Canada (10), France (14), Persian Gulf (3), Mexico (21), Italy (1), Sweden (43), United Kingdom (5), South Africa (16), West Germany (6), Pacific Ocean (30), Egypt (13), Vietnam (7).

CREATIONISM IN TEACHING

Small changes, big fuss

San Francisco

AFTER months of public debate, the California Board of Education last week approved new curriculum guidelines that give evolution a more central place in science education. But some last-minute concessions to creationists led to very different accounts of the decision's significance in national and in regional news.

At issue were changes in the wording of the science framework that were negotiated in the past few weeks to guarantee the board's approval. The board vote attracted widespread national attention because California controls about 11 per cent of the country's elementary and junior high school textbook market, and its curriculum guidelines — to which publishers must conform — are considered to be influential in setting national standards. The guidelines also influence high school texts, even though the state does not develop an approved list.

The new framework will take effect from 1992 as part of a curriculum overhaul that California undertakes every seven years. Its implementation follows months of public debate over the place of creationism in the science curriculum, which culminated in a public hearing last October.

After that hearing, some board members insisted on several changes to appease Christian fundamentalists. A sentence was deleted that declared that "there is no scientific dispute that evolution has occurred and continues to occur; this is why evolution is regarded as a scientific fact." And phrases were added to the guidelines stating that "some people reject the theory of evolution purely on the basis of religious faith" and that these "personal beliefs should be respected and not demeaned". These changes were heralded by both the *New York Times* and ABC television news as an important victory for creationists, but the *San Francisco Chronicle* referred to the revised guidelines as "a blueprint for teaching science that emphasizes evolution and relegates creationism to social science classes".

But both sides in the dispute agreed that the last-minute changes were relatively minor. Dan Chernow, chairman of the state's curriculum commission, said that while he was disappointed at the changes, he believed that they are "meaningless". And a spokesman for the California-based Traditional Values Coalition said the group was glad for the modifications but called them "crumbs". The revision leaves largely intact repeated references to evolution's fundamental importance to science. For example, the last six words were added to the statement that "the particular case of 'creation science' or 'scientific creationism' has been thoroughly studied and rejected by the leading scientific societies as qualifying as a scientific explanation". Left unaltered is the declaration that "evolution is the central organizing theory of biology, and has fundamental importance in other sciences as well. It is no more controversial in scientific circles than gravity or electricity is."

Such statements significantly stengthen previous guidelines, which made it clear that evolution must be taught but offered no hints to teachers or school officials on dealing with its role in the controversy surrounding creation theory. The new document addresses this shortcoming, noting in part that teachers "should make it very clear that from the scientific perspective, evolution, like other scientific topics, does not bear on an individual's religious beliefs. Science is not theistic, nor is it atheistic; it does not presuppose religious explanations." Chernow said creation theory should be discussed along with other religious issues, either in the history and social science curriculum or the English and language arts curriculum.

Robert Buderi

ANTARCTIC ASTRONOMY

Cerenkov telescope for the South Pole

Washington

PHYSICISTS from Purdue University and the University of Wisconsin at Madison will be travelling south next month to begin a three-year project that will see construction of the first optical telescope, albeit an unconventional one, at the South Pole. An array of ten 1-metre mirrors will be set out on the ice to look for flashes of light caused by ultra-high-energy particles shooting through the upper atmosphere.

For a number of years, researchers from the University of Leeds and the Bartol Institute at the University of Delaware have been operating an air-shower detector at the South Pole (see *Nature* 340, 192; 1989). This device registers the cascade of debris created by a high-energy particle hitting an atom in the upper atmosphere. The Purdue–Wisconsin group will use an alternative detection method which searches for the Cerenkov radiation emitted by a charged particle moving through a refractive medium such as air.

According to James Gaidos of Purdue, the first goal of the Antarctic project is to demonstrate that optical-quality mirrors will work successfully in the extreme climate. The prototype mirrors for the Purdue–Wisconsin telescope are backed with heating elements to keep their surfaces a few degrees above the air temperature, which should prevent condensation and fogging.

David Lindley

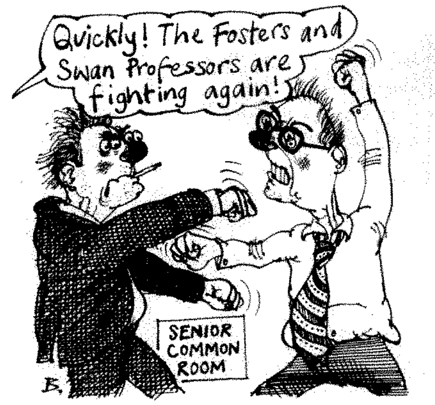
INDUSTRIAL SPONSORSHIP

Research for sale

Sydney

A DISPUTE at Melbourne University over a proposal for commercially sponsored professorial appointments has cast academic freedom and industrial needs as enemies. The university intends to create a new category of position by which private companies would select researchers and finance a university chair for them. The idea follows the recommendation of a report from a working party headed by the university's vice chancellor, David Penington, which says that corporate-funded appointments are necessary because the university has run out of money to create professorial appointments in new fields.

The sponsored professorial fellows would be appointed at the request of the donor company, without being subject to competitive selection. Nevertheless, Penington says that "for each corporate-backed professorial chair the University council must be satisfied that there are no strings attached. If a company wants to donate funds to the university to take on a particular individual, we would be foolish to appoint anyone else. We will, however, make sure of the calibre of the appointee." But the Federation of Australian University Staff Associations (FAUSA) is unhappy with the university's proposed safeguards. FAUSA believes that people appointed at the donor's behest may be reluctant to participate in research that the donor does not approve.


Quickly! The Fosters and Swan Professors are fighting again!
SENIOR COMMON ROOM

"If we give up academic independence and criticism for the money these corporations will bring in then the price is too high", says Ralph Hall, president of FAUSA. But Penington disagrees: "We must develop links with industry if research and development in Australia is to flourish."

Tania Ewing

Correction

Dr Gerrit Viljoen, the South African Minister for National Education, was professor of Greek at the University of Pretoria before becoming rector of the Rand Afrikaans University in Johannesburg (*Nature* **342**, 8; 1989). ☐

Freedom to conduct research

Sir—The near coincidence of the publication of letters from Donald Kennedy (president of Stanford University; *Nature* **341**, 180; 1989) and Norman R. Saunders of Southampton (*Nature* **341**, 99; 1989) prompts us to write. The former was in regard to Alex Comfort's claim that academics generally show cowardice in the face of external attack, while the latter dealt in part with the inappropriate comments of Clive Hollands (*Nature* **339**, 248; 1989) about a paper from the INSERM group on development of the primate visual system (*Nature* **337**, 265–267; 1989). We were beginning to think that Colin Blakemore (*Nature* **339**, 414; 1989) was the only scientist in Britain with the courage to speak out on an issue of fundamental importance to all scientists: namely, the freedom to conduct research without unwarranted obstruction or meddling. The reticence of scientists on both sides of the Atlantic to speak out in response to attacks by antivivisectionists such as Hollands tends to confirm Comfort's assertion.

We, too, question the propriety of a member of Britain's Animal Procedures Committee (APC) passing moral judgement on research he apparently knew little about in such a public forum as the Correspondence pages of *Nature*. At the very least, Hollands' credibility in terms of being seen as fair-minded in carrying out his duties on the APC has been compromised. Further, Hollands' decision to identify himself in the letter as a member of the Committee for the Reform of Animal Experimentation is troubling, and raises the question: is it appropriate for members of animal research 'reform' groups to serve on committees that regulate animal research? Do we really want foxes minding our research henhouses?

We do not question the necessity of regulating animal research, nor do we question the desirability of having as members of regulatory committees people who are truly concerned about animal welfare. Indeed, everyone who works with animals in any way should be concerned with their welfare, and happily the vast majority of scientists are so concerned. Our apprehension lies with empowering 'false moderates' who cloak their true antivivisection/antiscience agenda with euphemisms. Is this paranoia? We think not. As you point out in your leading article (*Nature* **339**, 491; 1989), conditions for and treatment of animals in research laboratories are improving, in part because of the clamour made by animal activists, many of whom claim to be concerned only with improving welfare. But the hypocrisy of this claim is well illustrated by recent experiences of our university and nearby Stanford. Both universities have been making great efforts at considerable cost to replace antiquated research facilities with state-of-the-art laboratories that will greatly improve the animals' environment. Nevertheless, these changes have been met by the animal 'welfare' advocates not with appreciation but with lawsuits and ever-increasing pressure to halt construction. It seems that every new regulation, stipulation or increased cost of doing animal research is viewed by the antivivisectionists as a victory, and causes them to redouble their efforts to halt such research by whatever means they choose to use. For the 'moderates', it means more lobbying for more expensive and restrictive regulations; for the extremists it means vandalism, theft, arson, bombs and death threats (so far). The silence of the vast majority of scientists on this issue may ensure successs for the antivivisectionists, and once again, in the words of Dr Mary Putnam Jacobi when she testified in 1900 against a bill that would have curtailed animal research in the District of Columbia, we shall see "the domination of knowledge by ignorance".

Charles S. Nicoll
Sharon M. Russell

Department of Integrative Biology,
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Sir—The US Department of Agriculture (USDA)'s "dramatic about-turn" allowing research institutions "themselves to decide how to meet the required standards" of animal care (*Nature* **341**, 6; 1989) will add to the already substantial abuse of animals in laboratories. As it is, USDA officials rarely inspect laboratories more than once a year, if that. Violations of the Animal Welfare Act often go undetected, and if they are reported, there may be no follow-up to ensure compliance with the law. Animal protection organizations have exposed blatant violations and abuse that would otherwise still occur.

USDA's authority over laboratories needs to be strengthened, as does the Animal Welfare Act. Allowing researchers to police themselves will result in conditions that benefit the research budget, not the animals.

K. S. Guillermo

People for the Ethical
Treatment of Animals,
PO Box 42516,
Washington, DC 20015, USA

ECU too

Sir—Fred Hoyle (*Nature* **341**, 380; 1989) finds that the term ECU (European Currency Unit) induces pictures of infinities of little grey men in a very large grey building in his mind. If he respells it, he will find the image quite changed.

The word "ecu" sounds to me like something small, hard and shiny (for example money). It is pronounceable in every European language (with the obvious exception of Greek, which does not have a "c"), and it is short enough to require no further abbreviation.

Provided it does not mean anything foul or ridiculous in any European language, why look further?

Andrew Jenkins

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Nature conservancy

Sir—As the British government's proposals to split up the Nature Conservancy Council (NCC) will undoubtedly generate much public debate during the next 18 months or so, I should like to correct some inaccuracies in Ben Webb's recent article (*Nature* **341**, 94; 1989).

First, NCC has no statutory responsibility for archaeological sites. Its remit covers the conservation of flora and fauna as well as features of geological or physiographical importance.

Second, before 1973, the then Nature Conservancy did carry out research, but following the formation of NCC this function was handed over, in part only, to the Institute of Terrestrial Ecology (ITE). But NCC's Chief Scientist's Directorate (CSD) coordinates or conducts a wide range of research and survey work necessary for NCC to exercise its diverse functions throughout Great Britain. To say that those opposing the government's proposed reorganization are unlikely to "seek to put the clock back to when NCC had a research capability of its own" is misleading, NCC utilizes such a research capability today. Indeed, the CSD provides the science base and overview upon which many areas of the NCC's work are justified and built.

Third, while Webb focuses on the need for plans to encourage farmers to take into account environmental considerations, no mention is made of other land uses and development pressures. The forthcoming debate will by no means linger on the farming community; many of the problems of conservation in Great Britain today are caused by other socio-economic factors related to industrial and residential development, transport and infrastructure provisioning, forestry and recreational pressures on seminatural and other habitats of importance for wildlife.

Peter J. Ewins

Nature Conservancy Council,
Archway House, 7 Eastcheap,
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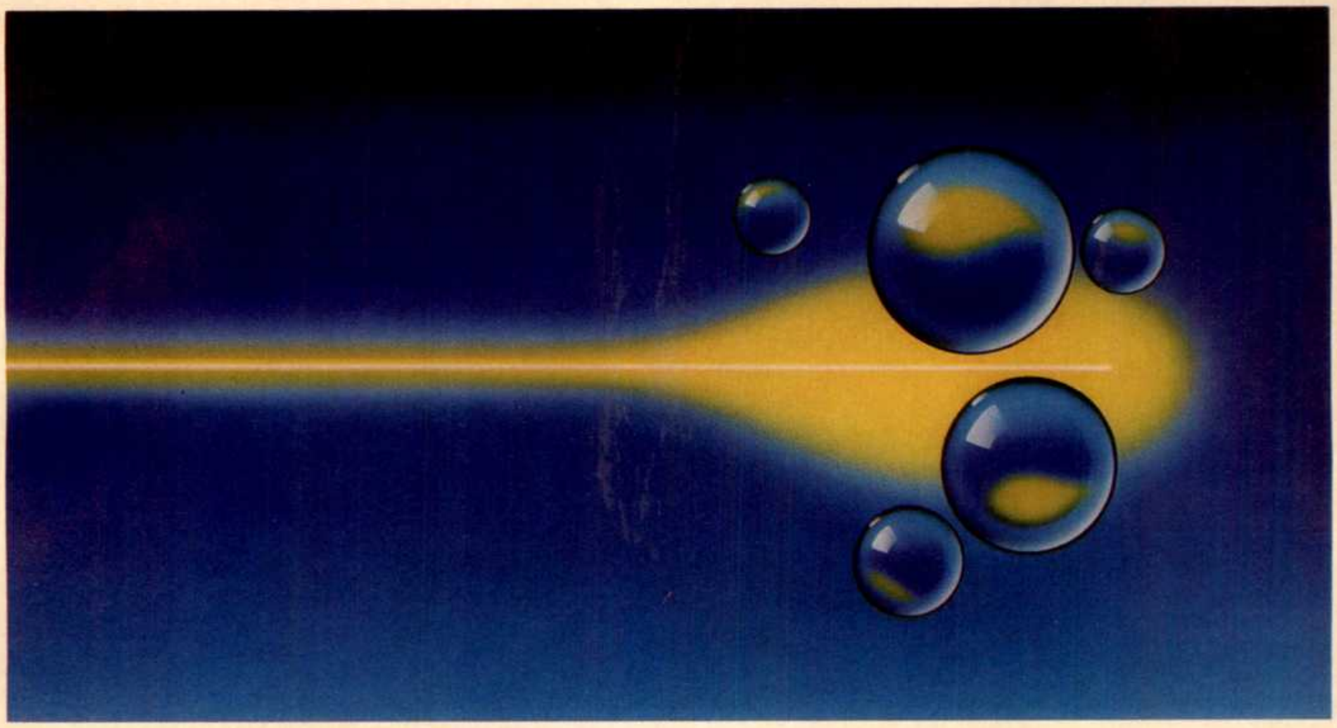

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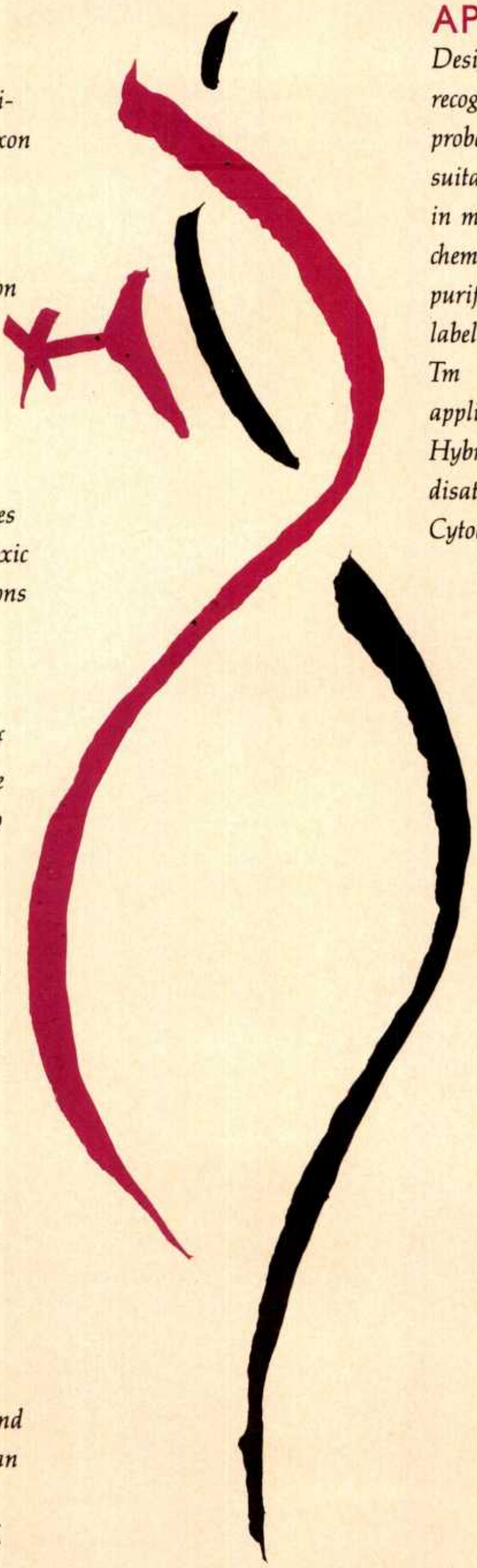

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MOLECULAR GENETICS

False start on manic depression

Miranda Robertson

MOST of the presentations at *Nature*'s conference in Boston, held last week, were a startling reflection of the pace of progress in the understanding of the genetic mechanisms of complex processes. The one conspicuous reversal, candidly acknowledged by Ken Kidd (Yale University), was in the genetics of psychiatric disease.

Readers of *The New York Times* may already be aware that the linkage reported in *Nature* two years ago[1] between manic depression and a locus on chromosome 11 is now acknowledged probably to have been spurious. The events that led to this conclusion are described, by a group including many of the authors of the original report, on page 238 of this issue[2], and were discussed at the meeting by Kidd who is co-author on both papers. At the end of his presentation, David Baltimore (Whitehead Institute) voiced the question that must have been left in many people's minds: "Setting myself up as an average reader of *Nature*", he asked, "what am I to believe?". What follows is an attempt to enable readers to answer the question for themselves.

A picturesque feature of the original study was the choice of the Amish community of Pennsylvania for analysis. The Amish are no more prone to psychiatric disease than other groups, but they have three advantages as subjects for the investigation of complex genetic traits. First, they have large families whose members do not disperse. Second, the restrictive rules they live by reduce confounding environmental variables such as drugs and drink. Third (and crucially), the Amish are relatively inbred, which increases the probability that an abnormal phenotype running in a family will be traceable to the same gene in all affected members.

The claim of the 1987 paper was that manic depression in a large Amish pedigree was linked to two marker genes, the *Harvey ras* oncogene and the insulin gene, known to be in the same region of the short arm of chromosome 11. Neither of these genes was itself implicated in the disease, nor was any other gene actually identified as the cause of the disorder: the claim rested upon the consistent inheritance of specific and recognizable variants of the two marker genes with the propensity to develop manic depression, and on a calculation of the probability that this co-inheritance could occur purely by chance. The traditional measure of this probability is the LOD score (LOD standing for the log-of-the-odds ratio), and the traditionally acceptable LOD value is 3, corresponding to a probability of 0.05 against the chance occurrence of the observed pattern of co-inheritance. The LOD score for the linkage of a chromosome 11 locus to manic depression was comfortably more than 4.

For any disorder, the assessment of linkage is complicated by the possibility of recombination events that may separate the marker from the gene and that become more likely the greater the distance between the two (this distance being of course unknown). For psychiatric disorders the calculation is further complicated by the likelihood of incomplete penetrance, which means that some individuals with the predisposing gene will fail to develop the disorder. Both factors must be taken into account in calculating linkages to psychiatric diseases.

Thus the conclusion that a gene on chromosome 11 could predispose to manic depression was calculated on a delicate balance of uncertainties. Two developments have now upset that balance. First, two people in the original Amish pedigree have become manic depressive in the absence of the putative markers of predisposition; second, an additional branch of the original pedigree shows negative evidence of linkage. This could mean that there are two genes instead of one predisposing to manic depression in the pedigree; but the simplest explanation is that the apparent linkage to a chromosome 11 locus was in fact just chance.

According to Kidd, chance may also account for a second genetic linkage, more recently reported by Hugh Gurling's group[3] which has published evidence implicating a locus on chromosome 5 in predisposition to schizophrenia. The conclusion in that case was based on two markers which, as Lander[4] remarked at the time, were uninformative for many members of the five families analysed, where the same marker variants were carried by both the affected and the unaffected parent.

In an attempt to find a marker that will distinguish the parental chromosomes in these cases, Gurling has since tested a third chromosome 5 marker which, as it turns out, shows less evidence of linkage than either of the other two. This could mean that the third marker is more distant from the putative schizophrenia locus than the others, allowing more recombination between the marker and the gene; but in fact Kidd maps the third marker in between the other two, ruling out that explanation. This leaves us with no persuasive evidence linking any psychiatric disease to a single locus and brings us back to Baltimore's question.

It is neither necessary nor justified to conclude from these reversals that there simply is no single gene capable of predisposing some individuals to psychiatric disease. Studies of identical twins have already provided very strong evidence, especially in the case of schizophrenia, for an important genetic component. There is on the other hand every reason to expect the predisposing genes to be hard to identify, for reasons that can be illustrated by the remarkably successful recent attempts at analysing the complex multifactorial genetics of predisposition to coronary heart disease.

The work on cholesterol metabolism in particular was discussed by Gerd Utermann (University of Innsbruck), who has been investigating the effects of plasma lipoprotein polymorphisms on coronary heart disease in people already known to be at risk through a mutation in one of their low-density lipoprotein (LDL) receptor genes. People who are homozygous for such mutations never escape heart attack. Heterozygotes on the other hand may have heart attacks by the age of 40, or they may be quite unaffected. What Utermann has been able to show is that polymorphisms in plasma proteins that affect cholesterol levels can account for much of the variation in effect of the LDL receptor mutation on heterozygotes.

In this case several interacting genes affecting the uptake and disposition of lipids can be seen to contribute to the risk of heart disease in such a way that no one mutant gene or polymorphic variant would reliably be inherited with the disease. We may expect the genetics of psychiatric disease to be at least as complex as those of cholesterol metabolism. But in the absence of a good biochemical clue to what genes may be involved, they will be much harder to trace. The hope has been that the high incidence of specific psychiatric disorders in inbred families reflects the existence of a single, strong, predisposing allele. If in fact it is necessary to seek linkage to more than one gene in such families, there are techniques that would make this possible[5]; but a high-resolution linkage map of polymorphic markers for the human genome would be needed for a reasonable chance of success, and in the view of many this should be a priority for the human genome project.

In the meantime, readers of *Nature* are entitled to be sceptical about reports of psychiatric disease linkages at LOD scores of less than 6. But they should have no reason to doubt the existence of genetic predisposition to psychiatric disease, nor the ability of molecular geneticists eventually to identify the genes responsible. □

Miranda Robertson is Biology Editor of Nature.

1. Egeland, J. A. *et al. Nature* **325**, 783–787 (1987).
2. Kelsoe, J. R. *et al. Nature* **342**, 238–242 (1989).
3. Sherrington, R. *et al. Nature* **336**, 164–170 (1988).
4. Lander, E. S. *Nature* **336**, 105–106 (1988).
5. Lander, E. S. & Botstein, D. *Cold Spring Harb. Symp. quant. Biol.* **LI**, 49–62 (1986).

STATISTICAL PHYSICS

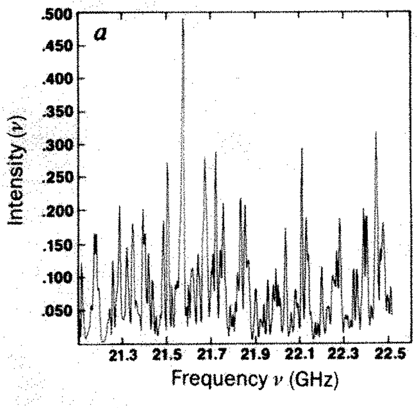
Microwaves in random media

J. B. Pendry

A DIFFICULT statistical problem, as yet unsolved, concerns the motion of waves in random media. The problem can be compared with the kinetic theory of gases, one of the great achievements of physics. Newton's equations of motion were redeveloped to formulate equations describing the macroscopic properties of the gas. Crucial to the process was the interplay between theoretical insight and experimental stimulation. In the case of waves in random media experimental results will be equally critical. A recent experiment by N. Garcia and A. Z. Genack (*Phys. Rev. Lett.* **63**, 1678–1681; 1989) provides a veritable paradigm of the problem for theorists.

It is no accident that the kinetic theory of gases was developed long before theorists felt able to tackle waves in random media. A large sample of gas can be characterized by macroscopic quantities such as temperature, pressure and volume, which are well defined and whose statistical fluctuations can be neglected. Not so for waves in a random medium: the further waves penetrate into a disordered system the more extreme become the intensity fluctuations. This is true for any degree of disorder; but for strong disorder, the wave is essentially scattered within its own wavelength and the resulting wavefield is even more statistically complex. The fluctuations cannot be ignored and must be included in any theory.

An example of these fluctuations is given in Garcia and Genack's article. The sample consists of uniform half-inch-diameter polystyrene spheres with filling fraction 0.56, contained in a 7.3-cm-diameter copper tube. The wavelength of microwave radiation used was about 1 cm. The spheres fill the tube uniformly, but can still move when the tube is tumbled, providing another statistical configuration. Microwaves are launched from a horn 20 cm in front of the sample and detected by a Schottky diode mounted on a plunger that defines the length through which the microwaves pass. Figure 1*a* shows the transmitted intensity as a function of frequency for a static sample 140 cm in length. Note the extreme fluctuations, which take the form of a series of sharp peaks separated by deep troughs. Tumbling the tube generates a different set of peaks and troughs, but of the same general character.



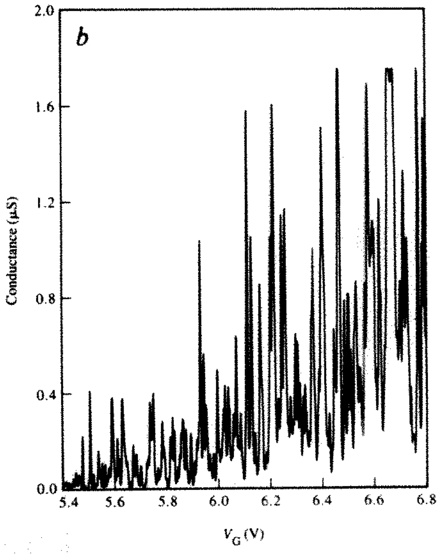


FIG. 1*a*, Spectral intensity fluctuations for microwaves passing through a 140-cm length of tube filled with half-inch-diameter polystyrene balls. *b*, Conductance of a silicon-metal-oxide field-effect transistor as a function of gate voltage measured at 50 mK.

If these results were unique to microwaves the experiment would be a mere curiosity. Let us take a couple of other examples from the pages of *Nature*. A few months ago in News and Views (*Nature* **339**, 581; 1989), I discussed waves in semiconductors: in that instance the waves were, of course, electrons. When the semiconductor is disordered, the conductance–voltage plot may look like that shown in Fig. 1*b*. These data (A. B. Fowler, J. J. Wainer & R. A. Webb *IBM J. Res. Devel.* **32**, 372–383; 1988) are for a silicon-metal-oxide field-effect transistor at 50 mK. The similarity in form to the microwave transmission experiments is striking because the origin of the fluctuations is the same: waves in random media.

An earlier example comes from W. H. Munk and G. O. Williams (*Nature* **267**, 774–778; 1977). This paper discussed the transmission of 406 Hz acoustic signals through the ocean. The distances in question were very long — of the order of 1,000 km — and the disorder results from mesoscale fluctuations in the ocean's currents. A simulation of the acoustic transmission through a typical path, plotted in the complex plane (Fig. 2), reveals both amplitude and phase fluctuations. It is clear that extreme fluctuations are again involved. A related phenomenon is the fading of radio waves in the ionosphere. An example closer to common experience is provided by the propagation of a thunder clap across a random landscape: close to the strike a single sharp crack is heard; farther away the Fourier components in the crack pass through the random acoustic filter provided by a disordered landscape, and recombine to produce the characteristic rumble whose long duration bears witness to the narrow bandwidth of the transmission peaks.

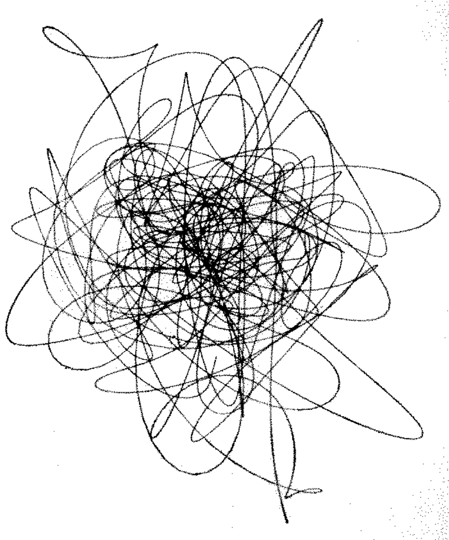


FIG. 2 Acoustic transmission simulated for transmission through approximately 1,000 km of fluctuating ocean at a frequency of 406 Hz. The distance from the origin shows the intensity; the angle, the phase.

Of these examples, the electronic instance is the most important, with its consequences for problems likely to be encountered in the construction of small low-dimensional devices. Despite the rich variety of experiments on disordered electronic systems, there are some problems involved in comparing them with the current imperfectly developed theories. First, the scale of electronic wavelengths is such that probes on this scale have yet to be developed. Second, electrons are mutually interacting particles and the complication of treating disorder at the same time as electron–electron interactions is one that the theorist would rather be without until a clear understanding emerges of the effects of disorder alone. Third, electronic experiments are, as a rule, made on a single device. Switching to another device is often time consuming and it is impossible to perform ensemble averaging over specimens. This can be a great disadvantage where strong fluctuations are important.

The microwave experiment eliminates all these objections and should prove an ideal test bed to knock on the head unsuccessful theories, and to help good ones.

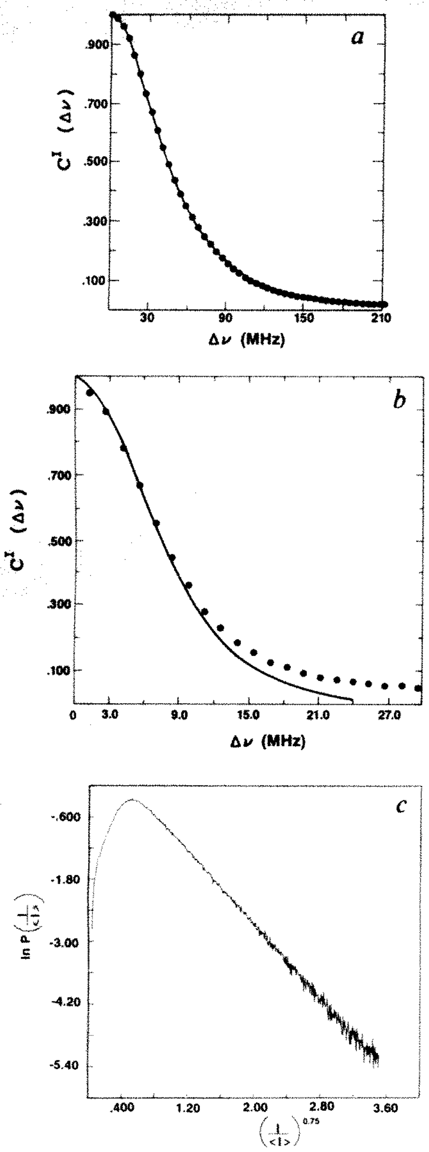


FIG. 3 The correlation function $<I(\nu)I(\nu+\Delta\nu)>$ for transmitted intensity at two frequencies separated by $\Delta\nu$. The solid line shows the theoretical fit. *a*, Length of tube is 30 cm; *b*, length of tube is 140 cm. In the longer sample, strong-disorder effects begin to assert themselves and the correlation function shows a high-frequency tail not explained by the theory. *c*, The probability distribution of intensities transmitted through a tube of length 140 cm, plotted against $(I/<I>)^{3/4}$.

Figure 3 provides an instance of this process at work. It shows intensity-intensity correlation functions from Garcia and Genack's paper where the intensities are measured with frequency separation $\Delta\nu$. Theory developed for the weak-scattering limit fits the data very well for short samples (Fig. 3*a*), but not for longer ones in which the scattering builds up to large values (Fig. 3*b*). Another puzzle is shown in Fig. 3*c*, a plot of the probability distribution of transmitted intensities. A curious exponent of 3/4 is found for the dependence of $\log(P(I))$ on I. So far theory does not have an explanation.

Theorists are not completely at sea with these problems. Even for strongly disordered samples we have a general understanding of why strong fluctuations occur. Disorder strongly inhibits the passage of waves through the medium, and therefore it is possible for resonant cavities to form at random within the medium. Waves can be trapped in these for a long time before tunnelling out. The longer they are trapped, the sharper the resonances, as we understand from conventional theory of resonances, so that a thick, strongly disordered sample will have more sharp resonances than a short, weakly disordered one. The resonances play an important role in the transmission properties of the sample: waves whose frequency is degenerate with a resonance show enhanced transmission. The sharp spikes seen in Fig. 1*a* betray the presence of resonant cavities within the sample. The statistics of transmission through strongly disordered samples is the statistics of these resonances.

A whole series of new experiments is opened up to us. What are the effects of exchanging the one-dimensional tube with a two-dimensional sheet? Can the reflection coefficient be measured? And how do the resonances beneath the surface affect the noise characteristics of the reflected signal? Can a more complex medium be studied, for example one in which the dielectric medium has lower symmetry and shows optical activity? What about the higher moments of the transmission coefficient? The answers to these questions are eagerly awaited. ☐

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PROTEIN STRUCTURE

Chaperones, paperones

Joe Sambrook and Mary-Jane Gething

FOR the past several years, chaperone proteins have been a valuable commodity for speculators in protein function. Identified in most organelles of eukaryotic cells, chaperones bind to newly synthesized polypeptides and apparently stabilize them until they are assembled into their correct native structure[1,2] or until they are transported into another cellular compartment[3]. With the description by Holmgren and Bränden on page 248 of this issue[4] of the first three-dimensional structure of a chaperone protein, albeit one that is bacterial and rather unusual in several respects, comes a result that was not predicted by any of the speculators: the two structural domains of the protein that seem to carry out the chaperone function resemble immunoglobulin domains.

Most of our knowledge about chaperones has come from biochemical and genetic studies carried out in eukaryotic cells[3,5-8]. As far as we know from these studies, chaperones are not true catalysts of protein folding. They are, for example, unable to catalyse the formation or isomerization of covalent bonds in the target proteins. In these respects, chaperones differ from conventional enzymes such as protein disulphide isomerase, prolyl isomerase or prolyl hydroxylase, that modify the structure of newly synthesized proteins. In addition, chaperones can remain attached to their target proteins for far longer than an enzyme would be expected to interact with its substrate[9]. Finally, many chaperones can interact with a wide variety of target proteins *in vivo*[1,2] and with synthetic peptides of diverse sequence *in vitro*[10]. These targets seem not to have any amino-acid sequences or structural motifs in common. Diversity on such a scale is not found in substrates for conventional enzymes.

Most of the experimental evidence is, instead, compatible with the idea that chaperones stabilize segments of polypeptide that might otherwise serve as local centres for intra- or interchain aggregation or denaturation. In this way, chaperones might provide the time needed for thermodynamic forces to drive folding of large and complex proteins to completion. According to this idea, chaperones would increase the efficiency of folding and assembly of newly synthesized proteins, but would not necessarily expand the repertoire of interactions that occur between polypeptide chains in the final protein. Alternatively, they might also maintain parts of the nascent polypeptide chain in configurations that could eventually be converted into a wider variety of shapes and surface than would otherwise be possible. Chaperones would therefore not only increase the efficiency of protein folding but would also extend the range of interactions both within and between polypeptides. A simple analogy is the lock and key structures that hold together poppet heads in a child's necklace. Although such interlocking structures are unknown in proteins, there are many examples of interactions between deeply convex and concave surfaces. Chaperones may serve as a mechanical jig to hold segments of a polypeptide chain in a state that allows complex surfaces to form. Perhaps, without chaperones, only a subset of complex topological surfaces could be generated by interaction between 'pre-assembled' polypeptides.

Holmgren and Bränden's crystal structure, which provides a new window on chaperone mechanisms, is of the PapD protein isolated from the periplasmic space of *Escherichia coli*. Strains of *E. coli* that cause nephritis carry special pili (aptly named P pili) that allow the bacteria to adhere to digalactoside residues on the surface of cells lining the urinary tract[11,12]. These pili, which project through the outer bacterial membrane and cell wall, consist of approximately 1,000 protein subunits and are assembled in the periplasmic space from components that have been translocated across the inner bacterial membrane. The shaft of each pilus consists of repeating units of a single protein (PapA), whereas the tip consists of three proteins, PapE, PapF and PapG[13], the last of which carries the digalactoside-binding activity of the pilus[14].

The single operon that encodes all of these proteins also encodes two accessory proteins that are not incorporated into the pilus but are required for its formation[12,15]. One of them, PapC, appears to form a channel in the outer bacterial membrane through which the growing pilus emerges[16]. The other, PapD, is a periplasmic protein that forms complexes with subunits of at least two of the proteins (PapG and PapE) before they are assembled into the pilus[17]. Earlier this year, a group of Swedish workers proposed that PapD acts as a chaperone to enhance the postranslational folding and assembly of components of the pilus[18].

Holmgren and Bränden now show that PapD is built from two globular domains, each consisting of an antiparallel structure formed from packed β-sheets. The topology of each domain is the same as that of the constant domain of the immunoglobulins. Whereas there is no significant amino-acid similarity between either of the two domains of PapD and the constant domain of immunoglobulins, the sequence of 239 amino-acid residues can be aligned with the N-terminal 256 amino-acid residues of the Leu-1 human lymphocyte differentiation antigen (also known as CD5). Of the amino-acid residues in the aligned sequences, 26 per cent are identical. The authors propose that PapD and CD5 form a special subclass of the immunoglobulin gene superfamily. Whether or not their speculation that the subclass was dispersed by horizontal gene transfer during evolution turns out to be true, their work provides dramatic evidence of the durability and utility of this type of protein fold.

Chaperone watchers will be most interested in the region of PapD that potentially interacts with its target proteins. The best candidate is the wide crevice between the two domains that contains an exposed hydrophobic patch flanked by basic and acidic residues. In addition, the region linking the two domains perhaps serves as a flexible hinge that can open up to accommodate target sequences of various sizes. The orientation of the target polypeptide within this site is not known, as the PapD molecules crystallized by Holmgren and Bränden are 'empty'. It would be interesting to find out whether PapD, like chaperones such as BiP and HSP70 (ref. 10), can bind oligopeptides of defined sequence and, if so, whether the binding is tight enough to allow crystallographic analysis of PapD-target complexes.

PapD is an unusual chaperone in several respects. It is smaller than most, contains no obvious ATP-binding sites and appears to be more specific in its function. Unlike BiP or other members of the HSP 70 family, which bind to a wide variety of target proteins and perform functions that are indispensable to the life of the cell, PapD binds only to polypeptides encoded by the *pap* operon. Therefore it is not necessary for the survival of *E. coli*, but only for its colonization of the urinary tract. Because the function of PapD is more specialized than that of other chaperones, the potential polypeptide-chain binding site formed between two immunoglobulin-like domains may differ in detail from those of chaperone proteins that fulfil more generalized functions in protein folding and assembly. There cannot, however, be too many ways to form clefts that can accommodate polypeptide segments of varying sequences. Holmgren and Bränden's structure, together with that of the class I major histocompatibility antigen[19,20], provide examples of two ways to solve this problem. It remains to be seen whether other chaperones use similar structural motifs. ☐

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1. Ellis, J. *Nature* **328**, 378–379 (1987).
2. Ellis, R.J. *Trends biochem. Sci.* **14**, 339–342 (1989).
3. Deshaies, R. & Schekman, R. *Trends biochem. Sci.* **13**, 384– 388 (1988).
4. Holmgren, A. & Bränden, C.-I. *Nature* **342**, 248–251 (1989).
5. Pelham, H. *Cell* **46**, 959–961 (1986).
6. Deshaires, R.J. *et al. Nature* **332**, 800–805 (1988).
7. Normington, K. *et al. Cell* **57**, 1211–1236 (1989).
8. Rose, M.D. *et al. Cell* **57**, 1211–1221 (1989).
9. Bole, D.G. *et al. J. Cell. Biol.* **102**, 1558–1566 (1986).
10. Flynn, G.C. *et al. Science* **245**, 385 – 390 (1989).
11. Norgren, M. *et al. EMBO J.* **3**, 1159–1165 (1984).
12. Normark, S. *et al.* in *Microbial Lectins and Agglutinins: Properties and Biological Activity.* (ed Mirelman, D.) 113–143 (Wiley Interscience, New York, 1986).
13. Lindberg, F. *et al. Nature* **328**, 84–87 (1987).
14. Lund, B. *et al. Proc. natn. Acad. Sci. U.S.A.* **84**, 5898–5902 (1987).
15. Lindberg, F.P. *et al. EMBO J.* **3**, 1173–1176 (1984).
16. Norgren, M. *et al. Mol. Microbiol.* **1**, 169–178 (1987).
17. Lindberg, F.P. thesis, Umeå Univ. (1987).
18. Hultgren, S.J. *et al. Proc. natn. Acad. Sci. U.S.A.* **86**, 4357–4361 (1989).
19. Bjorkman, P. *et al. Nature* **329**, 512–518 (1987).
20. Bjorkman, P. *et al. Nature* **329**, 506–512 (1987).

OZONE DEPLETION

A dent outside the hole?

Guy P. Brasseur

THE formation of an ozone hole inside the polar vortex over the Antarctic continent each spring (September to November) since the late 1970s has been a cause of wide concern. In a rather provocative paper on page 233 of this issue[1], Proffitt *et al.* suggest that, from August to September, a substantial amount of high-latitude ozone is also destroyed outside the Antarctic vortex, by unidentified chemical processes. This conclusion is reached by an indirect but elegant analysis of simultaneous measurements of ozone, nitrous oxide (N_2O) and reactive nitrogen species (abbreviated as NO_y) made by stratosopheric jet-borne experiments.

Following the discovery of the ozone hole, there were several coordinated measurement expeditions, including the 1987 Antarctic Airborne Ozone Experiment from which Proffitt *et al.* derive their data, in an attempt to elucidate the mechanisms responsible for the depletion. These have revealed that active ozone-destroying forms of chlorine are produced in the lower stratosphere by the conversion of relatively stable molecules such as HCl and $ClONO_2$ on the surface of ice particles in the stratospheric clouds formed in the polar winter (when temperatures drop below −85 °C). The concentration of chlorine monoxide inside the polar vortex during the Antarctic spring is found to be 100 times higher than at midlatitudes: this is sufficiently elevated to destroy much of the ozone in a couple of weeks.

The suggestion by Proffitt *et al.*[1] of early-springtime depletion outside the polar vortex is a surprise because no such mechanism can operate there. The first step in the analysis leading to their suggestion is to note that, outside the chemically perturbed region of Antarctica, there is a persistent linear (negative) relation between the concentration of N_2O and NO_y in the lower stratosphere. The source of nitrogen oxides in the stratosphere is the slowly reactive nitrous oxide, which is produced at the Earth's surface and transported into the stratosphere by air currents. The necessary conservation of nitrogen atoms inside a given air parcel, as the conversion from N_2O to NO_y proceeds, explains the relation between the concentration of these species. The relation, however, does not hold in chemically perturbed regions, where NO_y is efficiently removed by ice particles and by their subsequent sedimentation. Thus, by making use of this relation between N_2O and NO_y, it becomes possible to discriminate between air masses that have been affected by the perturbed chemistry in the region of the ozone hole and those that have not been perturbed.

In the second step of their analysis, Proffitt *et al.* calculate the concentration ratio of O_3 to NO_y along a flight path from California to Chile. If, in the lower stratosphere outside the polar vortex, both ozone and NO_y are not significantly affected by chemistry, this concentration ratio should remain fairly constant. In air masses unperturbed by polar chemistry, this concentration ratio is of the order of, or larger than, 300 at heights of 18 – 21 km at all latitudes, except south of 50° S where it is substantially reduced. The authors conclude that a significant chemical sink must exist in the region between this latitude and the boundary of the chemically perturbed region, located near 65° S. Losses are about 15 per cent in late August and as high as 30 per cent in late September. These losses were not isolated events but were found throughout the period during which observations were made.

These results are consistent with statistical analyses[2] of ozone data obtained in this region. In addition, comparisons between vertically integrated ozone concentrations measured between 1979 and 1988 by the total ozone mapping spectrometer (TOMS) on board the Nimbus 7 satellite also indicate substantial ozone reductions in the Southern Hemisphere outside the chemically perturbed region[3,4]. But, in this case, the depletion was attributed to the dilution in late spring of ozone-poor air originating in the region of the ozone hole. The results presented by Proffitt *et al.* indicate that, even before the polar vortex breaks up, and probably as early as August (before the formation of the ozone hole), an efficient chemical mechanism destroys ozone at high latitudes, south of 50° S. The possibility that ozone is destroyed during the Antarctic winter (as early as June) in the layer where polar stratospheric clouds are present, was recently suggested by Fiocco *et al.*[5].

Despite all this evidence, no definite conclusion can yet be drawn. The number of ground-based Dobson instruments, by which ozone is measured (and which are used to correct some of the satellite measurements), is too small for the data analyses to be sufficiently reliable. Furthermore, the quantitative values reported by Proffitt *et al.* are somewhat marginal and do not show unambiguously that the anomaly in the O_3/NO_y ratio is a manifestation of chemical destruction. The increased value of this ratio they observed over the tropics is not entirely explained.

But alternative explanations for the measurements by Proffitt *et al.* are readily discounted. For example, the vertical exchange of air parcels outside the vortex would alter the local composition. As suggested by the high temperatures and maximum ozone (and NO_y) concentrations observed near 60° S, air parcels should subside in this region. But the O_3/NO_y ratio increases slightly with altitude, so that exchange would give the opposite effect to the one reported. Alternatively, enhanced production of NO_y, which is caused by galactic cosmic rays with maximum efficiency at 15–20 km, would result in a reduced ratio of O_3/NO_y. But this process is confined to a region near the magnetic pole that does not extend out to 50° S.

A chemical process thus seems to affect ozone at high latitudes outside the region where the Antarctic ozone hole appears and before the hole is produced. Further evidence for such an effect as well as an explanation of the processes involved needs, however, to be presented before it can be claimed that a significant chemical ozone destruction is taking place in winter and spring. The concentration of active chlorine outside the vortex is not large enough to account for a 15–30 per cent ozone loss even with the sunlight available at these latitudes during winter, so it seems unlikely that the hole-type loss mechanisms account for the observed ozone destruction. Perhaps heterogeneous chemical processes on the surface of background sulphuric acid aerosols are responsible. Other possibilities are mentioned by Proffitt *et al.*. In any case, it will be interesting to see if similar anomalies in the O_3/NO_y concentration ratio are found in the high-latitude region of the Northern Hemisphere. ☐

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1. Proffitt, M.H., Fahey, D.W., Kelly, K.K. & Tuck, A.F. *Nature* **341**, 233–237 (1989).
2. Albruitton, D.L. & Watson, R.T. (eds) *Stratospheric Assessment of Stratospheric Ozone* (UNEP/WMO Report, in the press).
3. Atkinson, R.J., Matthews, W.A., Newman, P.A. & Plumb, R.A. *Nature* **340**, 290–294 (1989).
4. Lloyd, P. *Nature* **340**, 269 (1989).
5. Fiocco, G., Komhyr, W.D. & Fuà, D. *Nature* **341**, 346–347 (1989).

PALAEOENVIRONMENTS

No smoke without fire

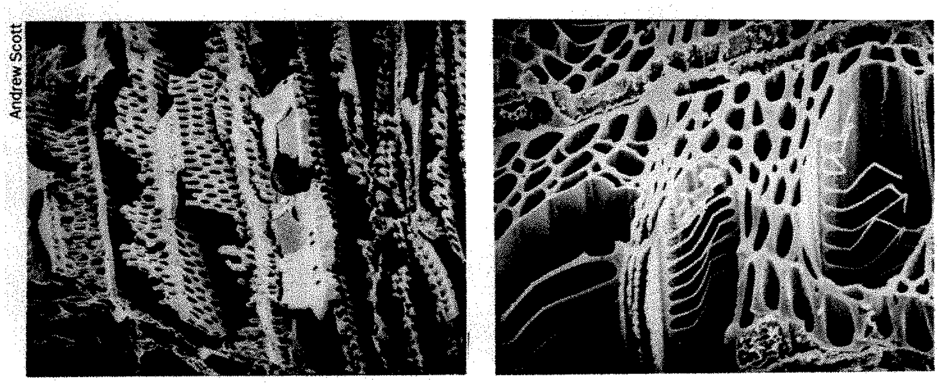
Peter D. Moore

AMONG her many other contributions to science, Marie Stopes, in 1919, analysed the constituents of coal and found particles of black, opaque matter that was chemically inert yet physically fragile. Stopes believed that this material, which she called 'fusain', represented fragments of plant charcoal derived from ancient fires. Fusain, which has subsequently proved to be a common component of coals and many other rocks, has attracted considerable attention in recent years as it could provide evidence of past wildfire frequency and hence of the gaseous and particulate composition of the palaeoatmosphere. Using scanning electron microscopy and reflectance microscopy to examine charcoal generated from contemporary plant material, Scott[1] has now laid the basis for further understanding the origin and significance of fusain.

The source of ancient fusain has been much debated. Some workers considered it to be a product of wet decay, but Harris[2] found that charcoal resulting from modern forest and heath fires bore a strong resemblance to fossil fusain. This observation led Harris to consider the role of fire in Tertiary times as an ecological determinant of vegetation, and as a selective factor in the evolution of Mesozoic flora. The dispersal of carbonaceous fragments through the air depends on settling velocity and hence on particle size. During a cruise across the Atlantic in 1980, Andreae[3] filtered fine particles of carbonized material (2–5 μm in size) from the atmosphere, and found that dispersal was so efficient that the fragment densities in remote parts of the Atlantic were comparable with those in rural continental areas. The presence of a 'soot' layer in clays from the Cretaceous/Tertiary boundary has subsequently raised the question of whether wildfire-generated smoke may have contributed to the change in climate that occurred during this period[4].

Cope and Chaloner[5,6] have used the presence of quantities of fusain in rocks as a basis for the reconstruction of palaeoatmospheres, claiming that, since the Lower Carboniferous, atmospheric oxygen levels cannot have fallen below 30% of their present level (a figure subsequently revised to 60%). This conclusion is based on the oxygen levels that would have been required to support the extensive forest fires that generated the widespread influx of fusain into sediments.

Scott[1] has now extended the experimental work of Harris[2] by scanning electron microscopy of carbonized material generated from a variety of plant parts, such as wood, leaves and flowers (see figure). He finds very close agreement between the experimental and the fossil material in the preservation of detailed anatomical structure of the tissues. Charred material often retains a clear and almost perfect structure, especially in wood samples but also in more delicate organs. Reflectance microscopy has also proved a valuable tool. Wood charcoal and wood-



Andrew Scott

Micrographs of, left, a fusain fragment of a pteridosperm axis from the Lower Carboniferous (270 µm across) and, right, modern beech (*Fagus*) charcoal (125 µm across).

derived fusain have very high reflectance values, whereas charred leaf tissues have lower reflectance. There is also a positive correlation between the temperature of combustion and reflectance. It should, therefore, prove possible to interpret fossil fusains with some degree of precision, both in the location of their source and their mode of origin.

Charcoal in coal is derived from either local wetland forest fires or from a considerable input of charcoal from airborne sources. The possibility of water transport into the site of sedimentation is unlikely for many coals because the coal-forming mires seem to have arisen as ombrotrophic (rain-fed) systems, similar to modern raised bogs, rather than rheotrophic (stream-fed) wetlands. Scott suggests that the origin of fusain could be determined simply by taxonomic analysis. Gymnosperm material is likely to mean upland fires and aerial input, whereas lycopod and fern fusain indicate local fire. The differential production and survival of carbonized particles from the different plants, must, however, be accounted for.

This line of research is promising both as a means of determining whether coal-forming mires were subject to fire (and, by implication, periodic drought), and also as a source of information on the ecological significance of fire in the evolution of plants and the development of vegetation.

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1. Scott, A.C. *Int. J. Coal Geol.* **12**, 443–475 (1989).
2. Harris, T.M. *J. Ecol.* **46**, 447–453 (1958).
3. Andreae, M.O. *Science* **220**, 1148–1151 (1983).
4. Wolbach, W.S., Lewis, R.S. & Anders, E. *Science* **230**, 167–170 (1985).
5. Cope, M.J. & Chaloner, W.G. *Nature* **283**, 647–649 (1980).
6. Cope, M.J. & Chaloner, W.G. *Nature* **290**, 428 (1981).

CHEMICAL SYNTHESIS

Scaling molecular Everests

John Mann

DURING a 2½ hour lecture in 1972, R. B. Woodward described the first total synthesis of vitamin B12. At that time it was the most complex naturally occurring compound that had succumbed to total synthesis. It had occupied the efforts of an army of chemists on two continents over a period of 12 years (R. B. Woodward *Pure appl. Chem.* **33**, 145–177; 1973; A. Eschenmoser & C. E. Wintner *Science* **196**, 1410–1420; 1977). In the audience that evening at Harvard was a young Japanese chemist, Yoshito Kishi, newly appointed to the staff of the Chemistry Department. He has now scaled his own 'chemical Everest' by synthesizing with his colleagues the structurally complex marine toxin, palytoxin (Y. Kishi *et al. J. Am. chem. Soc.* **111**, 7525–7530, 7530–7533; 1989).

Palytoxin, a product of marine coelenterates of the genus *Palythoa*, is the most toxic non-proteinaceous substance currently known. Its median lethal dose (LD50) is about 0.025 µg kg^{-1} for the rabbit and 0.45 µg kg^{-1} for the mouse. Although the LD50 for man is not known, the toxin was used in the past by native Hawaiians as a spear poison.

Palytoxin was first isolated by the group of R. E. Moore and P. J. Scheuer in 1963. They estimated its relative molecular mass to be around 3,000 (it is actually 2678.5), but the molecular formula of $C_{129}H_{223}N_3O_{54}$ remained in doubt until the gross structure was finally established in 1981 (Fig. 1*d*). An initial problem was classification of the toxin. Most natural products contain repeating subunits derived from acetate or mevalonate or other small building blocks, but such was not the case with palytoxin. The best one can say is that it possesses a highly functionalized fatty acid chain interrupted by monosaccharide residues.

In the mid-1970s, the Hawaiian group (Moore and Scheuer) and a Japanese group led by Y. Hirata used chemical degradation and the newly emerging NMR and mass-spectroscopic techniques to determine the gross structure of palytoxin. As the complexity of the molecule emerged, it became clear that the toxin presented an unprecedented challenge for the synthetic chemist. Kishi accepted the challenge.

First, his group synthesized (1980–82) each of the fragments produced by degradation, thus providing an unambiguous confirmation of the gross structure, and also complementing the stereochemical assignments being made (on the basis of spectroscopic studies) by the other groups. By 1985, they had devised practical routes to eight key building blocks, all in stereochemically pure form; the stage was then set for development of methods for the convergent coupling of these fragments. The successful completion of these efforts is the subject of the two recent papers, and Kishi now has in hand palytoxin acid which lacks only the terminal N-(3′ - hydroxypropyl)-3-aminopropenamide moiety of palytoxin.

There is no doubt that this synthesis could not have been accomplished in the 1970s. It required the revolution in the methods of asymmetric synthesis achieved in this decade, and the contemporaneous evolution of sophisticated two-dimensional NMR techniques to make it possible. The primary value of this latter

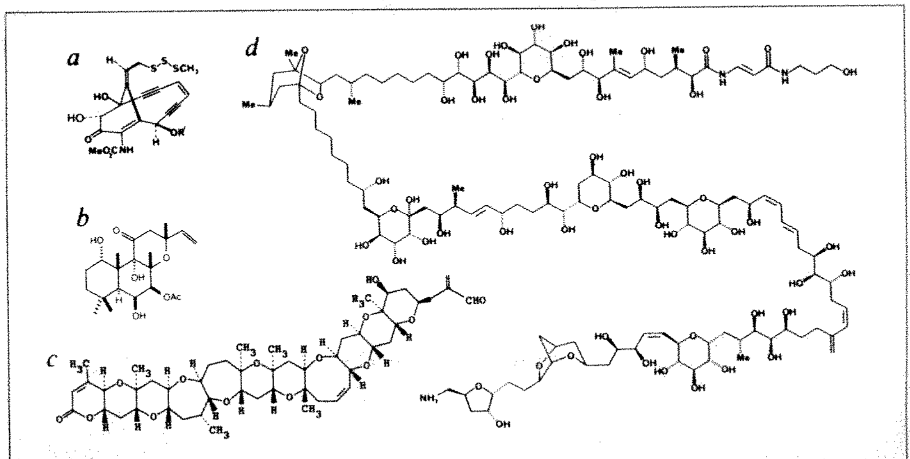

FIG. 1 Structures of palytoxin (*d*) and three other natural synthetic targets (see text).

to lower-crustal depths in the region). Their data also imply that the metamorphism occurred relatively recently — no more than 30 million years ago. Hayob *et al.* interpret the distribution of xenolith localities to indicate that the granulite-grade metamorphic event was regional in extent (at least 100 km^2 in area and perhaps extending for more than a thousand kilometres to the north). The heat source is inferred to be underplated basaltic magma, small volumes of which reached the surface as the xenolith-bearing basanites. The authors emphasize their evidence for the lack of a protracted history of cooling of the xenoliths (to temperatures less than 900 °C), and the fact that basaltic magmatism continues to be active in the region. They use these observations to postulate that granulite-facies metamorphism continues even now in southern North America.

Hayob *et al.* suggest that the lower crust can contain supracrustal rock types, that, in this case, the lower crust is granulitic, that granulite-facies metamorphism can be powered by mantle heat through basaltic underplating away from mountain-building (orogenic) settings, and that these processes are still continuing. None of these ideas or general observations (except for the extreme temperatures and limited retrograde cooling) is uniquely that of the authors. Their contribution lies in documentation and in the recognition of the collective importance of the separate pieces of data. The authors' arguments need some strengthening, particularly by the application of different age-dating techniques to xenolith mineral assemblages and integration with structural, tectonic and geophysical observations. But even as it stands, the evidence is sufficiently strong to make waves in the sea of geological opinion.

In the short term, the debate is unlikely to settle down. Granulite-facies metamorphism does occur over a broad range of conditions (700–1,100 °C, 400–1,500 megapascals, water activity 0–0.7) in varied tectonic settings. This suggests that a great variety of materials and processes are involved. The task ahead is to determine which are actually involved and which are most important. I expect that the underplating mechanism, combined with partial-fusion reactions in the absence of fluids, will turn out to be a principal factor in granulite-facies metamorphism, growth and differentiation of the crust, and the origin of upper-crustal granitic rocks. In an even broader context, I believe that core–mantle boundary instabilities, mantle plumes, anomalous conductivity in the lower crust, continental rifting, flood basalt outpourings and major biological events will all eventually come to be linked with these lower-crustal processes, operating on supracrustal rocks, in the same complex, discontinuous chain of events. ☐

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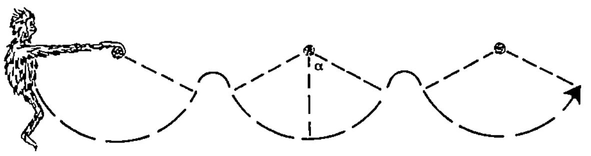
BIOMECHANICS

Gibbons swing stress away

R. McNeill Alexander

A FORCE acting on the end of a bone, parallel to its long axis, sets up stresses that are uniform across each cross-section. A force at right angles to the bone exerts bending moments on it, setting up tensile stresses in one face of the bone and compressive stress in the opposite face. When mammals run, the stresses in their bones are mainly due to bending moments. But when gibbons brachiate, swinging from branch to branch by their arms, the stresses in two of the principal arm bones are tensile right across the shaft, according to measurements reported by Sharon Swartz and her colleagues on page 270 of this issue[1]. By attaching strain gauges to the bones of captive gibbons and recording, by radiotelemetry, the strains during brachiation, they found that the humerus and ulna experience almost axial tensile loads. The radius, however, experiences bending moments because it is curved and is pulled on by the muscles that hold the fingers hooked over the branch.

A given force sets up much larger stresses in bones (or other long, slender structures) if it acts at even quite a small angle to the shaft than if it acts precisely axially. Gibbons can make do with remarkably slender arm bones because they load them axially: the humerus, for example, is 1.7 times as long as predicted for typical primates of the same body mass, but barely thicker than predicted[2].



Compare a gibbon of mass m and arms of length l, with a biped of the same mass and with legs also of length l. Suppose that the gibbon reaches out to a branch that is level with its shoulder and starts swinging. In the initial downswing it loses height l and gains speed v. The lost potential energy is converted to kinetic energy, so $v = \sqrt{2gl}$. The gibbon reaches this maximum speed in each swing, but its mean speed over a complete cycle of brachiation depends on the angle of swing, α (see figure). For $\alpha = 90°$, the mean speed is $0.5\sqrt{gl}$ (calculated as in ref. 3); for small values of α, it approaches $1.4\sqrt{gl}$. The force transmitted by the arm at the bottom of each swing is the weight plus the centripetal force, $mg + (mv^2/l) = 3mg$. If the arm is kept straight, the load on the bones is axial.

Our theoretical biped could maintain the axial load on its bones by keeping the supporting leg straight, as walking people do, but the axial load would be compressive rather than tensile as in the gibbon arm. Long leg bones could not be very slender or they would buckle under this load. Also, a biped using a straight-legged walk could not travel faster than $\sqrt{gl}$ because higher speeds would require downward accelerations greater than gravitational g (ref. 4). To travel faster, the biped would have to run, bending the legs at the knee and exposing the bones to bending moments. The peak force on a human runner's foot is about $2.8\,mg$, almost the same multiple of body weight as on the hand of our theoretical gibbon, but our theoretical biped would need leg bones thicker than the gibbon's arm bones to withstand the bending moments.

What would happen if the gibbon and the biped evolved longer limbs? By the argument already presented, the gibbon's speed would increase in proportion to the square root of its arm length. Similarly, the biped would increase speed in proportion to the square root of its leg length if it maintained dynamic similarity of gait[5]. In both cases the forces on the limb would remain unchanged, but the bending moments would increase in the biped in proportion to leg length. The longer-armed gibbon has no need for its bones to be thicker but the longer-legged biped does need thicker bones.

Gibbons are not limited by the speed they can gain in the first swing, but can accelerate by using leg movements like a child 'pumping' a swing[6]. They could accelerate faster by bending and extending the supporting arm instead of the legs, at appropriate stages of the swing, but that would result in bending moments. The penalty for relying on slender tensile arms is poor acceleration.

Finally, the really bad news, gibbon bones often get broken[7]. ☐

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1. Swartz, S. M., Bertram, J. E. A. & Biewender, A. A. *Nature* **342**, 270–272 (1989).
2. Aiello, L. C. *Symp. zool. Soc. Lond.* **48**, 331–358 (1981).
3. Preuschoft, H. & Demes, B. in *The Lesser Apes* (eds Preuschoft, H., Chivers, D. J., Brockelman, W. Y. & Creel, N.) 96–118 (Edinburgh University Press, 1984).
4. Alexander, R. McN. *Am. Sci.* **72**, 348–354 (1984).
5. Alexander, R. McN. & Jayes, A. S. *J. Zool. Lond.* **201**, 135–152 (1983).
6. Fleagle, J. *Nature* **248**, 259–260 (1974).
7. Schultz, A. H. *Bull. Hist. Med.* **7**, 571–582 (1939).

EVOLUTION

Parasitological teeth for evolutionary problems

Paul H Harvey

EVOLUTIONARY biologists have much to gain from a closer association with parasitologists It is not just that parasites have been overlooked for too long as a selective force, and that a considered assessment may show them to be at least partly responsible for a few of the problems that Darwin left us — sex, sexual selection and sociality have received attention recently There is more to it than that A parasitological perspective may shed light on evolutionary problems that do not necessarily involve one species parasitizing another Graham Bell and Austin Burt's assessment of the evolutionary significance of B chromosomes, which was presented at a recent symposium on evolutionary approaches to parasitism*, nicely illustrates the point

B chromosomes are distinguished from A chromosomes both by differing in number between individuals of the same species and by being unnecessary for the survival or reproduction of the individuals that possess them They are extremely common, probably being present in 10–15 per cent of eukaryotic species, and are typically small Often they are extensively heterochromatized — pointing to genetic inertness Even though they may have originated from the autosomes or sex chromosomes of the lineage in which they are found, B chromosomes have clearly evolved properties of their own In several plant species, B chromosomes are lost from the root tissue but retained in the aerial parts And, in the growing shoots, they may be lost from tissues that will become nonreproductive, yet increase in numbers in tissue that will give rise to the germ line Similarly, whereas small univalent chromosomes and chromosome fragments tend to be lost during meiosis, B chromosomes may actually accumulate In organisms ranging from grasshoppers to rye grass, the B chromosomes move preferentially during meiosis to those daughter nuclei that will give rise to germ cells rather than polar bodies or nongenerative nuclei

Given this accumulation of B chromosomes in the germ cells, why do their numbers not increase from generation to generation? The answer seems to be that they reduce the darwinian fitness of their hosts Cells with more DNA take longer to divide, and at least one study demonstrates that B chromatin adds about twice the amount of time to a typical cell division compared with a similar mass of A chromatin Morphological comparisons from a number of plant and animal species indicate that individuals with more B chromosomes usually exhibit delayed development, which is often accompanied by reduced vigour and fertility In other words, B chromosomes bear all the hallmarks of successful parasites

According to R N Jones and H Rees (*B-Chromosomes*, Academic, London, 1982), the parasite theory for B chromosomes was suggested as long ago as 1945 by G Ostergren Since then it has largely gone out of fashion and B chromosomes have been viewed as either of neutral selective value, or as being advantageous to the individuals or species that bear them The parasite theory, however, does in fact seem successfully to accommodate recent observations What is more, the theory makes fairly straightforward predictions that remain to be tested For example, if B chromosomes are disadvantageous to their hosts, they should be lost from clonal lines and asexual species In Bell and Burt's terminology, B chromosomes constitute not merely a disease, but a sexually transmitted disease

The parasitological perspective on B chromosome evolution may lead to the reinterpretation of other observations For example, there seems to be additive genetic variance for the presence and number of B chromosomes, which might be interpreted as differential resistance by particular sets of autosomes to invasion by B chromosomes If that is so, the 'Red Queen' theory for the evolution of recombination (selection in response to ever-changing environmental challenges) might be invoked that is, we might expect individuals possessing B chromosomes to increase recombination among the autosomes, so that new genetic variants that are resistant to B chromosomes would be more likely to arise At the same time, we should expect successful B chromosomes to reduce the rates of recombination in their hosts

So far, the evidence bearing on these questions, as reviewed by Bell and Burt, shows no consistent patterns Working out concise predictions, suitable comparative tests and experimental protocols are tasks for the future But to ask whether the work will be carried out by parasitologists or evolutionary biologists depends on an outmoded distinction between scientists with identical perspectives ☐

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* British Society for Parasitology Autumn Symposium City University London 29 September 1989 Organized by A E Keymer and A F Read

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DAEDALUS

Earth, wind and fire

SOME time ago the 'hydrogen economy' was proposed as a way of avoiding pollution Hydrogen would be made in bulk by the nuclear-powered electrolysis of water or, perhaps, by the chemical transformation of oil A universal fuel, it would burn cleanly to harmless water

This green dream soon faded into well deserved oblivion Nuclear power has its own potential for environmental disaster, and the generation of hydrogen from oil liberates as much greenhouse-inducing carbon dioxide as simply burning the oil in the usual way But Daedalus is now reviving the idea His plan is to turn the oil into hydrogen while it is still in the ground

The modern steam-reforming catalytic hydrogen process would be hard to employ on crude oil *in situ* Steam, heat and catalyst would all have to be fed continuously down the borehole But Daedalus recalls the old partial-oxidation process which burnt crude oil in a limited supply of pressurized oxygen with a little steam in it, giving mainly hydrogen and carbon dioxide No catalyst or added heat was needed So Daedalus plans to pump oxygen and water down a test oil well, ignite the mixture electrically at the bottom and collect the resulting hydrogen from a central exit pipe

Deep in the Earth, the process will run under great hydrostatic pressure Carbon dioxide and undesirable by-products will dissolve in excess condensed water, and the pressurized solution will soak back into the porous oil-bearing rock as fast as the oil flows out of it So the nasties will stay underground, only hydrogen will be volatile enough to escape to the surface

Geohydrogen would be tricky to distribute Overland, it could travel through the pipelines that now take oil and gas But as bulk sea-freight it would be troublesome Daedalus proposes to tow it round the world in captive balloons

A small tug could easily tow a big balloon full of hydrogen Even better, the balloon might tow the tug The wind direction often varies markedly with height, and by winching the balloon up to the right altitude, the tug could be pulled in any chosen direction — a wonderful advance on the traditional sailing ship Once discharged at its destination, the deflated balloon could be stowed on board the tug for the journey back to the oil field

As a major fuel, hydrogen could be piped around like domestic or industrial gas It could provide heat, power and clean water simultaneously and economically, wherever they are needed, ideally by means of a fuel cell All potential pollutants would be left behind in the oil fields Future geologists, prospecting for what remains of the oil, may be puzzled to strike dirty soda water

David Jones

Mechanism of solid-state fusion

SIR—Experiments by Derjaguin *et al*[1,2] indicating that MeV neutrons were emitted during the destruction of heavy ice or monocrystals of LiD went almost unnoticed, whereas recent claims of 'cold fusion'[3,4] have attracted worldwide attention, ranging from highly enthusiastic confirmations to equally sceptical rejections Here we wish to present some relevant considerations of mechanisms which could enhance nuclear interactions in the solid state

A body of experimental evidence[5,6] suggests that hydrogen resides in transition metals as a simple 'lattice gas' Although the rate for nuclear fusion of d + d in the lattice may be increased over the 'free' rate because of screening of the repulsive Coulomb potential by lattice electrons, the enhancement cannot be large enough[7] to explain the claims of refs 3 and 4 Likewise, single-neutron tunnelling at low energies (the Oppenheimer–Philips mechanism[8]) will not do the job, and an excited resonance state in ^{4}He (ref 9), because under these conditions the tunnelling time is much longer than the lifetime of the resonant state

Any enhancement of the fusion rate must then be 'chemical' in origin — the potential barrier between deuterons may change shape substantially, or deuterons may gain some unusual thermal activation

An increase in the density and effective mass of lattice electrons can cause a decrease of the shielding radius and a corresponding increase in barrier penetrability In addition, the combined effects of a redistribution of electrons and lattice deformation may produce a local minimum in the potential barrier Under the right conditions, a quasi-stationary complex can form, and an effect known as resonance transparency may appear[10] If such a complex has a vibrational frequency with an energy ~ 0 1 eV, the effective collision frequency of deuterons can be greater by as much as a factor of 10^9 over the simple 'lattice gas' value

A considerable increase in the fusion rate would also come about if a single deuteron were able to acquire ~ 10 keV of energy In a lattice in equilibrium such an energy transfer would not occur, but perhaps during the destruction of a lattice there might be significant localization of the destruction energy, which could be transferred to absorbed deuterons A macroscopic version of this effect is found in the electrostatic acceleration of deuterium ions in cracks, which, as noted by Cohen and Davies in their recent News and Views article[11], is apparently responsible for the appearance of neutrons during the shock destruction of D_2O and LiD (refs 1 and 2)

This is then a problem in piconuclear (pressure-induced) reactions rather than in thermonuclear reactions Common features may apply in the various experiments high overvoltages and D compression in ref 3, cold fusion in natural geophysical conditions in ref 4, and high static pressures and shears in refs 1 and 2

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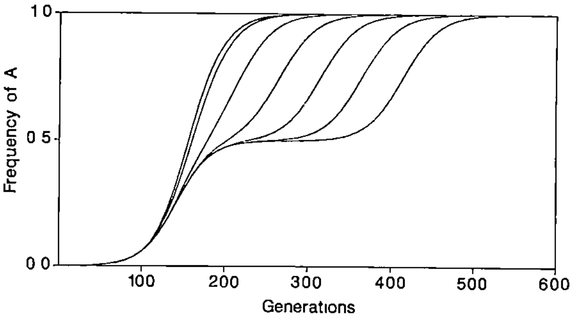
[1] Derjaguin, B V *et al* *Sov Colloid J* **48**, 12 (1986)
[2] Kluyev V A *et al* *Sov J tech Phys Lett* **12** 1333 (1986)
[3] Fleischmann M , Pons B S & Hawkins M *J electroanalyt Chem* **261** 301 (1989) and erratum, **263**, 187–188 (1989)
[4] Jones, S E *et al* *Nature* **338**, 737 (1989)
[5] Gel'd, P V *et al* *Hydrogen and Physical Properties of Metals and Alloys* (Nauka, Moscow 1985)
[6] *Hydrogen in Metals* (eds Alefeld, G & Volki, I) 2 vols (Springer Berlin, 1978)
[7] Baym, G & Leggett A J *Nature* **340**, 45 (1989)
[8] Oppenheimer J R & Phillips, M *Phys Rev* **48**, 500 (1935)
[9] Bevalacqua J *Phys Rev* C **27** 2417 (1983)
[10] Bohm, D *Quantum Theory* (Prentice-Hall, New York, 1952)
[11] Cohen J S & Davies, J D *Nature* **338**, 705–706 (1989)

Sex in diploids

SIR—Kirkpatrick and Jenkins[1] suggest that the origin and maintenance of sex in diploids is related to the advantage conferred by genetic segregation They contend that the segregation advantage gained by facilitating the fixation of advantageous mutations more than compensates for the twofold reproduction advantage of asexual reproduction We point out here that the segregation advantage they discuss is present even when only a very small proportion of individuals in a population reproduce sexually

To illustrate this, we have compared the pattern of increase in the frequency of a favourable allele in a population with only sexual reproduction to that in populations where there is a mixture of sexual and asexual reproduction[2] As an example, the figure shows the change in allelic frequency of advantageous mutation A when there is a 10 per cent selective difference between homozygotes and the heterozygotes are exactly intermediate in fitness Note first that the initial increase from 0 001 to nearly 0 5 occurs essentially at the same rate for all levels of sexual reproduction Furthermore, even if there is only a very small amount of sexual reproduction, the continued increase of the advantageous allele from 0 5 to fixation is nearly as fast as that in a completely sexually reproducing population



The change in frequency of advantageous mutation A for different amounts of sexual reproduction For the curve on the far right the proportion of sexual reproduction is 10^{-7} (1 in 10 million individuals reproduce sexually) Successive curves to the left are for increasing amounts of sexual reproduction (10^{-6}, 10^{-5}, 10^{-1}) The curve for 10 per cent sexual reproduction is indistinguishable from 100 per cent sexual reproduction

Kirkpatrick and Jenkins show that there is a plateau at a frequency of 0 5 for the heterozygous asexual population while it is waiting for the occurrence of a second mutation We observe a temporary plateau at an allelic frequency of 0 5 only when the proportion of sexual reproduction is about 10^{-6} or smaller This plateau, which is generally very short and in the most extreme case in the figure only doubles the time to fixation of the favourable mutant, occurs because the population consists of nearly 100% heterozygotes at the beginning of this period and is waiting for segregation to produce AA homozygotes (which it will at a rate about ¼ times the rate of sexual reproduction) We also find that the relative duration of this plateau is even shorter when the selective difference between the homozygotes is reduced

A number of species have a small (but not zero) amount of sexual reproduction[2,3] Perhaps this is a way in which a species can have both the segregation advantage discussed by Kirkpatrick and Jenkins and the twofold advantage of asexuality many others have noted In other words, we see why some sexual reproduction should be favoured over complete asexual reproduction because of the segregation advantage, but it is not clear why a lot of sexual reproduction should be favoured over a little[4]

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SIR—I believe that the mechanism proposed by Kirkpatrick and Jenkins[1] is unlikely to offer a sufficient explanation for the maintenance of diploid sex

Of critical importance in assessing the potential role of the segregation advantage[1] in maintaining diploid sex is the fact that the gradual accumulation of beneficial homozygous loci by a sexual population can only lead to gradual increases in its relative fitness Hence, a significant time period (Kirkpatrick and Jenkins provide an estimate of ~10^6 generations) would be required before the level of homozygosity in a sexual population could counterbalance the supposedly inherent twofold fitness advantage of asexual reproduction But throughout these many generations the asexual population would enjoy a large, slowly diminishing selective advantage, which should result in the competitive elimination of the sexual population long before its fitness approaches that of the asexual population In addition, competition with a more fit asexual population would lead to a continuous reduction in the size of the sexual population, slowing its relative rate of production of new beneficial mutant alleles, and further reducing its competitive prospects Even if the sexual population eliminated the asexual population, new asexual mutants, starting with the same genetic complement as the sexual population, would again enjoy a selective advantage for, say, ~10^6 generations In the absence of any advantage to sex other than an initially higher rate of fixing homozygous loci, the repeated appearance of such mutants would keep a sexual population at a constant disadvantage

The segregation advantage cannot explain the evolution of sex or its maintenance in haploid forms[1] However, the existence of sex in species with predominantly haploid life cycles implies a selective advantage sufficient to overcome any costs associated with meiosis Surely, parsimony supports a single explanation for the maintenance of sex in both diploid and haploid species rather than hypotheses exclusive to either group

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KIRKPATRICK AND JENKINS REPLY—We showed[1] that the process of genetic segregation can generate an evolutionary advantage to sexual reproduction that may help explain its prevalence over asexual reproduction Hedrick and Whittam point out that the segregation advantage we identified will be almost as effective in a population in which most individuals are asexual and only a small fraction are sexual Such a facultatively sexual population would have the best of both worlds most of the segregation advantage from sexual reproduction and most of the twofold reproductive advantage from asexual reproduction Species of this type, however, are virtually unknown among the animals[5], and so this may not be an evolutionary option (We do not consider automixis (selfing) here because the ubiquitous deleterious effects of inbreeding[6] probably select against it in most populations) We have extended Hedrick and Whittam's observation by considering the more common (though still rare) situation of cyclic parthenogenesis, in which the population undergoes a number of strictly asexual generations followed by a single generation of sexual reproduction[5] The results are similar to Hedrick and Whittam's an occasional generation of sexual reproduction is sufficient to give most of the segregation advantage and still retain most of the twofold reproductive advantage of asexuality

Why then are facultative and cyclic parthenogenesis uncommon? We have two possible explanations First, the segregation effect we identified is only one of the evoluntionary advantages to sex Others, such as effects from recombination[4,5,7], may work in conjunction with the segregation effect to give purely sexual reproduction an overall advantage Second, constraints may preclude facultative and cyclic parthenogensis as evolutionary options for many taxa[4,5] Pure apomictic parthenogensis has appeared dozens of times among the animals, but facultative and cyclic apomictic reproduction have arisen perhaps only half a dozen times[5] Thus, for most taxa the evolutionary possibilities may be purely sexual or purely asexual reproduction In this case, the benefits to sex from the effects of segregation and recombination may be sufficient to maintain sexual reproduction

Dunbrack reiterates two points made in our paper that there can be a substantial time lag before the segregation advantage offsets the twofold reproductive advantage of asexual reproduction, and that the segregation advantage will be greatly weakened when much or all of the genome is selected in a haploid state for a substantial part of the life cycle Dunbrack feels these points are fatal to our hypothesis We disagree based on the arguments presented in our paper, and re-emphasize that there need not be only one evolutionary advantage to sex — the segregation effect can be augmented by others

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1 Kirkpatrick, M & Jenkins, C D *Nature* **339**, 300 (1989)
2 Marshall D R & Weir B S *Heredity* **42**, 159–172 (1979)
3 Jackson, J B C *et al Population Biology and Evolution of Clonal Organisms* (Yale University Press, 1985)
4 Maynard Smith, J *The Evolution of Sex* (Cambridge University Press 1978)
5 Bell, G *The Masterpiece of Nature The Evolution and Genetics of Sexuality* (University of California Press, Berkeley 1982)
6 Charlesworth D & Charlesworth, B *A Rev Ecol Syst* **18**, 237–268 (1987)
7 Michod, R E & Levin, B R *The Evolution of Sex An Examination of Current Ideas* (Sinauer Sunderland 1988)

More on cold fusion

SIR—Processes involving cosmic-ray muons have been proposed[1–3] to explain the small rate of fusion neutrons observed[4] in the cold-fusion experiments Although fusion neutrons have not been observed in electrochemical cells placed in muon beams[5,6], the flux from the control experiment has not been measured Any cold-fusion neutrons of amuonic origin would remain unaffected by the muon beam These muon experiments therefore demonstrate negative results for cold fusion — both muonic and amuonic in deuterium-charged metals The muon experiments establish that the deuteron concentration in the samples does not reach abnormally high densities because the muons are lost to the more electropositive host-metal ions as expected for normal ratios of deuterons to metal-ions (up to two) Under conditions of high-density non-equilibrium deuteron packing as predicted by some of the cold-fusion experiments[7] and theories, the muon kinetics are not known, but loss to the host metal should be hindered, as it is known to be sometimes delayed[8] even in ordinary compounds because of intermediate molecular states

Known cosmic-ray muon fluxes[9,10], their stopping rates[11], and established muon-catalysed-fusion parameters[12,13] indicate that exposure of a large enough volume of liquid deuterium (about 1 litre) to cosmic-ray muons should also yield a low-level neutron count of about 10^{-2} s^{-1} There is a possibility of using these fusions for fissile-fuel breeding Although the rate would be extremely slow, the muons would be free and the muon-production cost factor could be dropped from the efficiency calculations for muon-catalysed fusion

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1 McCevoy A J & O Sullivan, C T D *Nature* **338**, 711 (1989)
2 Chatterjee, L Preprint JU HEP/89/2, Jadavpur Univ (1989)
3 Chaterjee, L *Proc ICENES 1989* (in the press)
4 Jones, S E *et al Nature* **228** 737–740 (1989)
5 Nagamine K *Proc Conf Muon-Catalysed Fusion* (Oxford 1989) (in the press)
6 Davies, J *Proc Conf Muon-Catalysed Fusion* (Oxford, 1989) (in the press)
7 Fleischmann, M & Pons, S *J electroanalyt Chem* **261** 301–308 (1989) and erratum **263**, 187–188 (1989)
8 Gerstein S S & Ponomaryev L in *Muon Physics* Vol 3 (eds Hughes, V W & Wu, C S) (Academic, New York 1978)
9 Hayakawa, S in *Cosmic Ray Physics* Vol 22 (ed Marshak R E) (Wiley Interscience, New York 1969)
10 YashPal & Peters, B *Nat fys med Dam Vit S* **33** (1964)
11 Wightman, A S *Phys Rev* **77** 521 (1950)
12 Jones, S E *Nature* **321** 127–133 (1986)
13 Chatterjee L *Phys Lett* A**137** 4–6 (1989)

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High-latitude ozone loss outside the Antarctic ozone hole

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Data taken during the 1987 Antarctic Airborne Ozone Experiment based in Punta Arenas, Chile, are used to show that from mid-August until the end of the mission in late September there was a high-latitude ozone loss outside the Antarctic ozone hole. Therefore, not only is the geographic extent of the ozone loss larger than that generally identified as chemically perturbed, but ozone is lost earlier in the year than previously reported. These results, when compared with long-term temporal trends of column ozone, indicate a possible anthropogenic component for this loss.

MEASUREMENTS from the past few years have shown that stratospheric ozone decreases substantially over Antarctica during September and October and that these decreases have intensified dramatically during the past decade. The intensification has been attributed to man's release of certain chlorine-containing compounds, primarily chlorofluorocarbons. The region of very high ozone decrease, usually referred to as the ozone hole, approximately covers the Antarctic continent, and is coincident with what is commonly called the polar vortex. Horizontally, the polar vortex is roughly defined by its perimeter, the polar night jet, a circumpolar wind belt in the stratosphere that seems to restrict exchange of air through the perimeter. The seasonal period of low ozone values is observed to terminate with the annual dissipation of the Antarctic vortex during the spring warming, at which time the air within the vortex is no longer restricted but allowed to mix freely with ozone-rich air from other latitudes. Transport towards the Equator and mixing of ozone-poor air with air at lower latitudes result in localized decreases of ozone[1]. The extent and intensity of these decreases are of global concern.

The seasonal decrease in ozone is large enough to provide an unequivocal signature of loss that is easily distinguished from natural short-term dynamical variability, and has been verified from the ground, aircraft, ballons and satellites. In this paper 'ozone loss' is used to identify a change in ozone due to chemical mechanisms (rather than dynamical processes) that is not predicted within standard gas-phase photochemistry models. We do not intend to imply the loss is necessarily due solely to chlorine. Measurements of total column ozone (total number of ozone molecules vertically above some area of the Earth) made from the ground and from a single station first identified ozone loss over Antarctica[2]. Satellite column measurements then verified that it was not just a local phenomenon[3]. Measurements obtained from balloons provided snapshots of the vertical distribution of ozone at a few Antarctic locations and presented striking accounts of large ozone decreases when comparing profiles from August with those from October[4,5]. But the changes observed were not simply a gradual deterioration of the ozone layer, but included a substantial day-to-day variability and frequent horizontal layering. This means that the region of decreased ozone is neither vertically nor horizontally homogeneous, and that quantification of the altitude-dependent decrease of ozone requires good temporal and geographic coverage. Although satellite measurements of total column ozone are frequently available, they cannot identify the altitude dependence of the ozone loss. *In situ* aircraft measurements can provide vertical profiles and extended horizontal coverage, but they are still insufficient to sample the entire geographic extent of the vortex frequently.

Here we present a different approach to estimating the ozone (O_3) loss inside and, more interestingly, outside the ozone hole—one that completely avoids the problems of sampling an inhomogeneous air mass. As standard gas-phase photochemistry is very slow in the region of this analysis (that is, during high-latitude winter in the lower stratosphere where O_3 has a photochemical replacement time of more than a year[6,7]), O_3 can be used effectively as a conservative tracer. The sum of the reactive nitrogen species (abbreviated as NO_y and including in that sum NO, NO_2, NO_3, HNO_3, N_2O_5 and $ClONO_2$) and nitrous oxide (N_2O) are also conserved in this environment. By combining *in situ* measurements of these tracers with empirically derived formulations that are supported by two-dimensional atmospheric models, minimum reference values for unperturbed O_3 can be estimated for any air parcel sampled. This reference value for O_3 can then be compared with the measured value, and the difference is a deviation from conservative behaviour that can be interpreted as a chemically induced change not within the standard gas-phase photochemistry. The formulations presented are first applied outside the region usually referred to as chemically perturbed. Then the analysis is used in a different manner, but this time both outside and inside. It is assumed for the analysis that there has been sufficient simultaneous sampling of the trace constituents NO_y, N_2O and O_3,

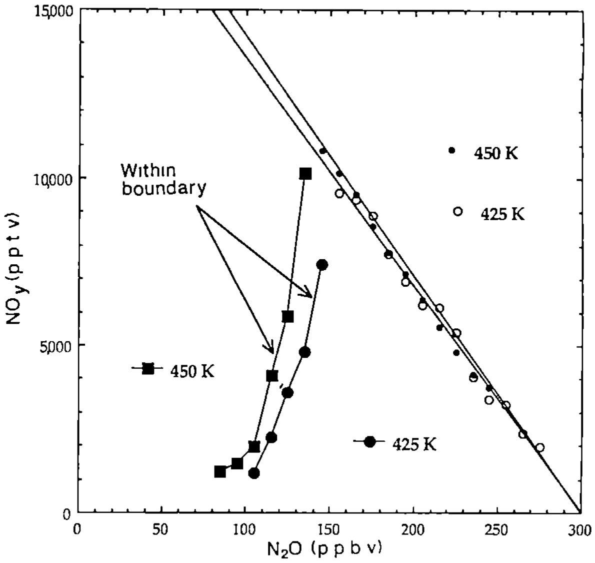

FIG 1 NO_y against N_2O from 1987 AAOE for the 425-K and 450-K flight levels and averaged for the seven flights where data are available over intervals of 1° latitude. Data taken within clouds were excluded to obtain only gas-phase NO_y. Linear least-squares fits to the data outside the boundary are shown for each flight level.

spatially and temporally, to determine reliable reference formulations.

To discuss accurately the morphology of the ozone hole, the concept of the boundary of the chemically perturbed region is used as introduced and developed by Proffitt *et al*[8] from data taken during the 1987 Antarctic Airborne Ozone Experiment (AAOE). The boundary, defined by a level of chlorine monoxide (ClO = 130 p.p.t.v. (parts per 10^{12} by volume)), effectively locates where this critical component in the catalytic destruction of O_3 by chlorine has a sharp poleward increase. It was found that there were also dramatic poleward decreases in NO_y and H_2O at this boundary, hence the basis for describing the interior region as chemically perturbed. We shall refine this description slightly by referring to this region as 'high chemically perturbed', and thereby not exclude a possible lesser degree of chemical perturbation outside the boundary. Proffitt *et al.* have shown that this boundary is slightly poleward of the peak wind speeds of the polar night jet and that it coincides with the latitude of the strong poleward decrease in total column O_3 as measured by satellite. In addition, the boundary locates the perimeter of the large isentropic temporal O_3 decrease (ozone hole) to within at least 1° of latitude (~111 km).

Danielsen and Houben[9] give heuristic arguments for wintertime diabatic cooling over Antarctica persisting into early spring, carrying ozone-rich mid-latitude air downward and poleward into the polar vortex. Proffitt *et al*[10] have presented supporting evidence from AAOE indicating a slow progression of diabatically cooling and descending lower statospheric air advectively spiralling poleward as it crosses the polar jet into the ozone hole. This view can be compared with that of Tuck[11], who also concludes that there is a significant mass flow through the vortex, but argues that it is the downward diabatic motion within and outside the vortex that is important. With either view, the region near and within the ozone hole is being slowly replenished with mid-latitude ozone-rich stratospheric air during the period of O_3 decrease through diabatic descent. If this is the case, temporal trends in measured O_3 will not indicate the total loss of O_3 in a particular parcel of air unless the time series begins before there is any loss and the measurements follow the descending parcel throughout the time interval of the trend[10]. This is a difficult, if not impossible, sampling requirement, even with precise knowledge of a parcel's descent path. Possible mixing of air parcels of dissimilar origin during or following the O_3 destruction process further complicates the sampling problem.

The analysis presented is based entirely on the *in situ* data taken during AAOE from the high-altitude ER-2 aircraft. During the mission the average latitude of the boundary was 66° S, but varied from 59° S to 71° S, its position responding to synoptic-scale meteorological forcing of the polar vortex. The data are generally restricted vertically to the two levels of 425 ± 10 K and 450 ± 10 K in potential temperature (corresponding to geometric altitudes ~17.5 km and 19 km respectively over Antarctica) and horizontally from 53° S to 72° S latitude at ~65° W longitude. The longitudinal coverage is quite restricted, extending only from the southern tip of South America to the region over or near the Antarctic Peninsula. Also, data were collected during the transit flights between Moffett Field, California (37° N) and Punta Arenas, Chile (53° S), where the twelve ER-2 Antarctic flights originated.

Lower limit for unperturbed ozone

The stratospheric source for NO_y is the very unreactive species N_2O. The primary destruction mechanism for N_2O is ultraviolet radiation at wavelengths ≤225 nm, has N_2 and $O(^1D)$ as principal products, and occurs primarily in the middle of the stratosphere above 30 km. A secondary loss occurs also in the middle stratosphere where NO is created in the reaction of $O(^1D)$ with N_2O, providing the primary source of NO_y. Plots of N_2O against NO_y outside the boundary show them to be linearly related (Fig. 1). These data are from the two flight levels of 425 K and 450 K of potential temperature and were analysed separately by averaging within 1°-latitude bins with respect to the boundary and over a one-month period. The data in Fig. 1 labelled as within the boundary (corresponding to $N_2O \leq 140$ p.p.b.v. (parts per 10^9 by volume)) are known to be from within the highly chemically perturbed region. Linear least-squares fits are shown for the data outside this region and it is apparent that the straight line that fits the data at the lower altitude (or potential temperature) also fits the data at the upper altitude rather well. The linear fit to all of the points in Fig. 1 outside the boundary is given by

$$[NO_y] = 20{,}800 - (69.8 \times [N_2O]) \qquad (1)$$

where the mixing ratio for NO_y is in p.p.t.v. and for N_2O is in p.p.b.v.[10,12,13] It is noteworthy that data taken during the flights from Punta Arenas to Moffett Field, where minimum values for N_2O of 210 p.p.b.v. were observed, also fall on the same straight line[10]. There are few further data available to verify the persistence of the linear relationship at other seasons, latitudes and altitudes, although the mid-latitude Southern Hemisphere balloon data taken by Galbally *et al*[14] up to 30 km have the same characteristic. This persistent linear relationship and lack of curvature results from a balance of photochemistry, transport

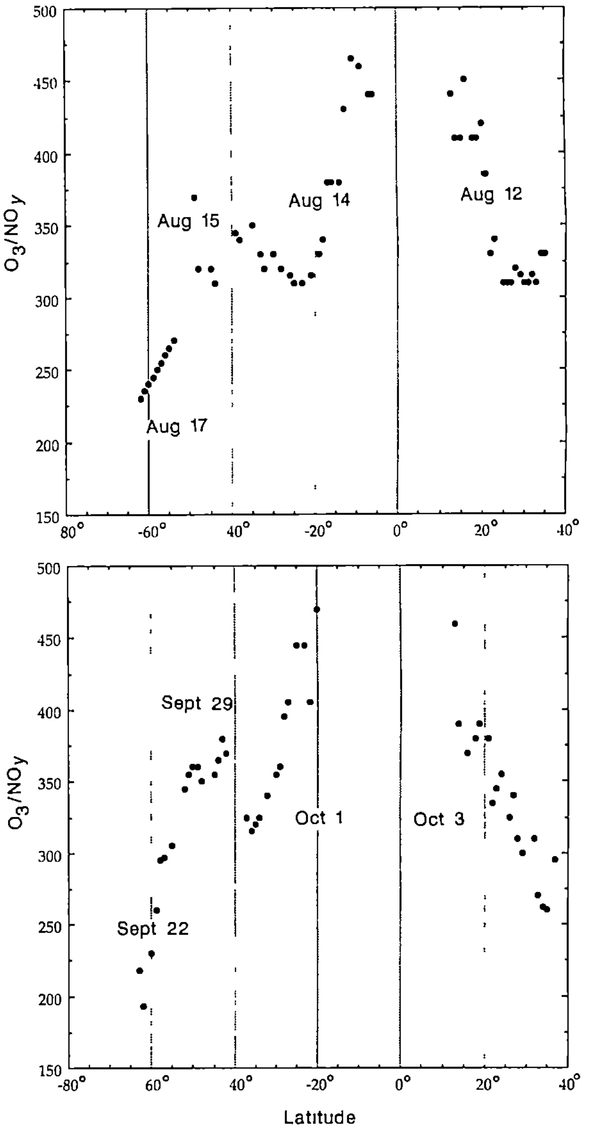

FIG. 2 O_3/NO_y latitude survey from *a*, 12 August to 17 August, 1987 and *b*, 22 September to 3 October, 1987.

and mixing. The N_2O intercept and slope are probably determined in the tropics where upper tropospheric and lower stratospheric air with N_2O of ~300 p.p.b.v. and NO_y <0.5 p.p.b.v. (intercept) rises into the tropical middle stratosphere where NO_y is produced from N_2O apparently with an average efficiency of ~7% (slope). Mixing, as the middle stratospheric air proceeds poleward, probably contributes to the observed small variability from linearity observed at high latitudes.

The ratio of O_3 to NO_y (O_3/NO_y) can now be used to bring O_3 into the analysis. Figure 2*a* is a plot of O_3/NO_y against latitude taken at the start of the mission and includes data taken in transit from Moffett Field to Punta Arenas. The ratio is >300 over a wide range in latitudes, but decreases at the most southerly latitudes. Figure 2*b* is a similar latitude survey including the last Antarctic flight and the transit flight data during the return to Moffett Field at the end of the mission. Still lower values at the southernmost latitudes and a few data points below 300 at about 35° N are the primary differences between the two surveys. Both figures are restricted to pressure altitudes from between 18 km and 21 km, and all data within the boundary are excluded. In both plots O_3/NO_y >300 at all latitudes north of ~55° S, except on the flight of 3 October over California, where a few values as low as 260 are found. Both surveys also show an enhanced ratio in the tropics where, at 20 km, stratospheric air is apparently encountered that had recent O_3 production without the equivalent NO_y production required for the usual ratio found at mid-latitudes. Other latitude surveys of ER-2 data taken during the Stratosphere Troposphere Exchange Project in January and February 1987 from 40° N to 30° S have been analysed (D. M. Murphy *et al.*, manuscript in preparation) and show a minimum for the ratio of 280 when restricting the data to potential temperatures >430 K. Also presented by Murphy *et al.* are two-dimensional model calculations[15,16] and satellite data[17] in the tropics and mid-latitudes covering pressures down to 7.5 mbar. Both the models and the data show that the O_3/NO_y ratio is >300 in the lower stratosphere and indicate either no vertical change or a slight decrease with altitude. Qualitatively these observations are consistent with the well-known mid-latitude mixing ratio maximum for O_3 and NO_y at 30–35 km with the maximum for NO_y a few kilometres higher than the O_3 maximum. These maxima correspond to N_2O values of 30–100 p.p.b.v., values much less than those measured outside the boundary and, as seen in Fig. 1, generally less than any data from AAOE.

From the observations made during ER-2 latitude surveys it is reasonable to assert that if NO_y and O_3 are given in identical units (such as p.p.b.v.) the inequality

$$[O_3]/[NO_y] \geq 270 \qquad (2)$$

is representative of late winter in the Southern Hemisphere north of the boundary. The data and model calculations indicate that a larger value of 300 for the minimum ratio could be justified. But there is no established record of simultaneous measurements of NO_y and O_3 covering all latitudes, seasons and altitudes of interest from which unequivocal formulations relating O_3 to NO_y could be derived. So the conservative approach taken here is appropriate, although it may underestimate the O_3 losses that have occurred.

Ozone losses

On 17 August between 54° S and 63° S (Fig. 2*a*), there was a significant difference between the expected O_3/NO_y ratio (inequality (2)) and its measured value. As NO_y was not perturbed and O_3/NO_y is less than our reference level of 270, an O_3 loss is indicated in that region. The value for O_3/NO_y of 230 at 63° S yields a 15% loss of O_3 outside the highly chemically perturbed region on 17 August. Data from the flight of 23 August also indicates a 15% loss outside the boundary, and the flights of 28 August and 22 September show a larger loss of 30%. But rather than giving flight-by-flight analysis, Fig. 3 shows averages for the period 23 August to 22 September to summarize the results and demonstrate that the O_3 losses are not isolated events found on certain flights, but were prevalent throughout the period and clearly seen in the averages. This is achieved by referencing the flight-leg data for NO_y and O_3 relative to the boundary, and then averaging the O_3/NO_y ratio over bins of 1° latitude. Only data for which NO_y is believed not to be enhanced by particles (as indicated by simultaneous particle measurements) have been plotted. This averaging procedure is done independently for both of the flight levels. The vertical bars represent the sample standard deviation and indicate how flight-by-flight variability affects the results.

The abrupt increase south of the boundary is due to the high degree of denitrification within the highly chemically perturbed region[13] and provides little information on the O_3 loss. We deal below with this region in a different way. However, outside the boundary where the NO_y is known to be unperturbed relative to N_2O, there are depressed ratios even below 200 near the boundary. These low ratios correspond to an average minimum O_3 loss of 15–30% in the first 5° of latitude outside the highly chemically perturbed region at these levels. It should be noted that the averaging over the one-month period is in a region where diabatic descent is believed to be taking place. An important feature in Fig. 3 is that outside the boundary the ratios at the 450-K level are ~20% higher than those at the 425-K level. This is not what would be expected from the model calculations and satellite data mentioned above, which show either no change in O_3/NO_y with altitude or a decrease. The implication is that the lowest values seen near but outside the boundary could not have come simply from vertical descent in this region. Proffitt *et al.*[10] show that within the averaging procedure used for Fig. 1, <10% of the air outside the boundary had come recently from the highly chemically perturbed region. Thus, if these results are accepted (that is, ongoing diabatic descent and little outward mixing at these potential temperatures) along with inequality (2), the decrease in the O_3/NO_y ratio, seen as the boundary is approached from the north, cannot be due to transport or mixing of air vertically nor horizontally, and therefore must be caused by some chemical mechanism operating at these levels outside the highly chemically perturbed region.

The isentropic temporal changes in O_3 outside the boundary

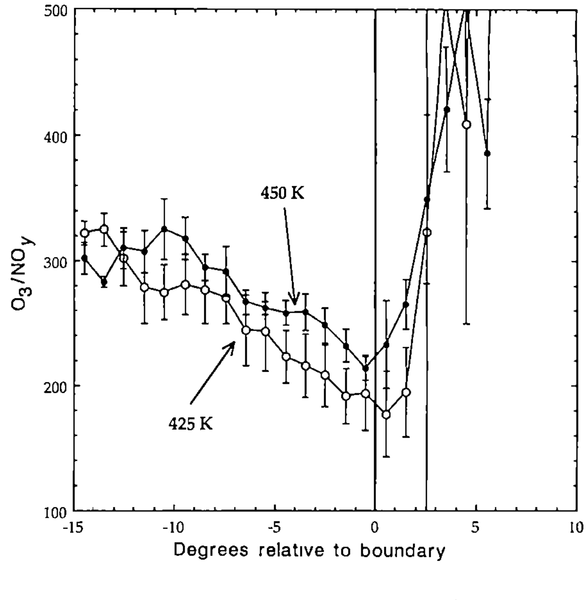

FIG. 3 O_3/NO_y against latitude relative to the boundary (heavy vertical line) for the 425-K and 450-K flight levels and averaged for the seven flights where data are available over intervals of 1° latitude. Data taken within clouds were excluded to obtain only gas-phase NO_y. Sample standard deviations are given as vertical bars.

during this one-month period are small in comparison with those inside, and in general are not significantly different from zero[18] As discussed in the introduction, losses of O_3 in a parcel of air undergoing diabatic descent will not be represented accurately by a temporal trend calculated at a constant potential temperature, because the losses will be masked by the usual vertical gradient in O_3 Therefore the lack of a significant isentropic temporal trend outside the boundary in no way indicates that no loss of O_3 is occurring there

To extend our analysis into the ozone hole and provide a check for consistency between two methods, equation (1) and inequality (2) are now combined into an inequality relating unperturbed O_3 directly to N_2O If O_3 and N_2O are both in ppbv

$$[O_3] \geqslant 5616 - (18\ 85 \times [N_2O]) \qquad (3)$$

provides a minimum value for unperturbed O_3 First note that this formula is based on measured data and may reflect systematic errors in the measurements In this regard, the O_3 measurement used here was generally within 2% of an independent measurement of O_3 made simultaneously on the ER-2[19,20] There was no independent check of the NO_y measurements except as they related to the O_3 data over a long history of more than 50 ER-2 flights, but they are reported to be accurate to within ±20% and repeatable (precise) to within at least 0 1 ppbv[13] The values used for N_2O are from the ATLAS instrument[21 22] and include a recent recalibration of the data that was not included in earlier papers[8 10-13 21,22] The reported accuracy of the N_2O measurements is ±10%

There is more uncertainty in applying inequality (3) inside the boundary where $N_2O < 140$ ppbv (see Fig 1), stemming from our lack of information to support equation (1) and inequality (2) at these more southerly latitudes Outside the boundary, equation (1) was used to test where NO_y was perturbed, then inequality (2) was used to predict O_3 By extending the application of inequality (3) to 72° S with its lower values for N_2O it is assumed that equation (1) remains linear and inequality (2) is still representative of O_3/NO_y The use of equation (1) at lower values of N_2O is supported by noting that the linear relationship between NO_y and N_2O is predicted to persist at mid-latitudes to at least 14 ppbv of NO_y in both a one-dimensional radiative convective photochemical model[23] and a two-dimensional model[16] Furthermore, the model calculations discussed earlier indicate that inequality (2) is satisfied at flight altitudes throughout our latitude range Therefore all information available supports inequality (3) for these low values of N_2O Implicit in the above discussion is that the formulations do have limits of applicability, the most obvious one being that they do not apply at altitudes above the maximum stratospheric mixing ratios for O_3 For at such altitudes, N_2O and O_3 are both decreasing with altitude but inequality (3) implies that as N_2O decreases, O_3 increases With this reservation a lower-limit estimate for unperturbed O_3 both outside and with increased reservation inside the boundary can now be calculated Figure 4*a*, *b* shows measured O_3 against latitude with respect to the boundary where O_3 is averaged over all ten flights from 23 August to 22 September at both flight levels Otherwise data are treated as in Fig 3 for O_3/NO_y Also plotted is the corresponding minimum value of unperturbed O_3 calculated from inequality (3) Figure 4*c* shows the O_3 loss with respect to the boundary, calculated as the percentage decrease in O_3 and based on Fig 4*a*, *b* The loss outside the boundary is typically 20-25% at 425 K for the first 5° with a maximum value indicated of ~30% From Fig 3 we obtained a maximum value of 30% O_3 loss outside the boundary, which is virtually identical to the loss shown in Fig 4*c*, although the analysis of Fig 3 includes only seven of the ten flights included in Fig 4 This difference in number of flights is because particle-free NO_y data are available only from seven flights Both analyses indicate that the loss is greater at the lower level, which is consistent with diabatic

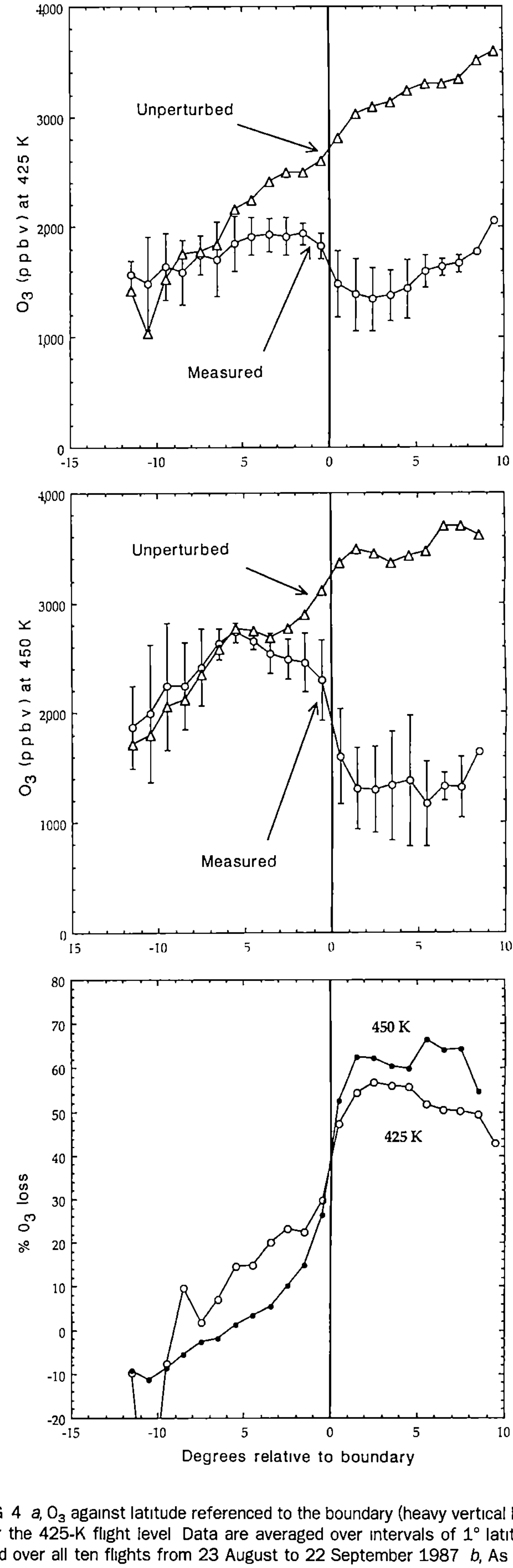

FIG 4 *a*, O_3 against latitude referenced to the boundary (heavy vertical line) for the 425-K flight level Data are averaged over intervals of 1° latitude and over all ten flights from 23 August to 22 September 1987 *b*, As in *a* except at 450-K flight level *c*, Average percent O_3 loss over 30 days derived from *a* and *b* descent and a simultaneous chemical loss of O_3

This analysis can also be applied to individual flights rather than averages of flights For example, from data taken on 23 August, the first day that a flight clearly penetrated the boundary, we calculate a 40% loss in O_3 inside the boundary On the last mission flight date, 22 September, still two weeks before the minimum column O_3 was measured in 1987 (ref 24), there was a 70-80% loss within the boundary in the layer between 17 5 km and 19 km This is substantially more than the 53% isentropic temporal decrease observed in the same region from 23 August to 22 September[18]

Discussion and conclusions

Since the ozone hole was first identified in 1985 it has been considered to be a recurring springtime event with a nearly monotonic increase of intensity over its history of ~10-15 years[25] Here we have presented strong evidence of significant O_3 loss occurring as early as mid-August both inside and outside the highly chemically perturbed region, and that this loss is chemical in origin rather than a result of atmospheric dynamics Although the mechanism for the chemical loss outside the boundary cannot be inferred from this approach, there is evidence of a temporal column O_3 decrease over the past decade in this region, therefore indicating that the chemical loss that we report has an anthropogenic component In particular, ref 26 shows, by comparing satellite data from 1979-80 with 1986-87, a nine-year temporal decrease from 6% to 18% in column O_3 between 60° S and 66° S during August and September, a region near, but on the average outside, the boundary during AAOE In ref 27 the data were analysed with a linear fit of the yearly data, starting with 1978 and ending with 1988 This analysis produced virtually identical trends from 60° S to 66° S from mid-August through September Both studies also show a long-term temporal decrease of more than 10%, poleward of 66° S during August These analyses present a consistent picture of mid-August O_3 decreases, both inside and outside the vortex, that have intensified during the past ten years The results we have presented co-locate a region of chemical O_3 loss with these long-term temporal decreases in O_3, suggesting that at least part of the loss has an anthropogenic origin

The cause of the O_3 loss outside the boundary, where the measured ClO is only 5-10% of the level measured inside the boundary, is not known, and more experimental investigation is warranted But it has been suggested by Tuck[11] that O_3 losses may occur near the boundary and outside the highly chemically perturbed region during June, July and August as long as there is sunlight Such losses would be due to reactive chlorine, as is the case within the ozone hole itself Support for this conclusion comes from photochemical model studies made along isentropic trajectories outside the boundary[28] These studies point out that the discrepancy between observed and measured values of ClO and NO indicates significantly perturbed photochemistry in this region without substantial denitrification In sunlight the formation of polar stratospheric clouds (PSCs) outside the boundary can perturb chlorine and nitrogen chemistry, initiating catalytic destruction cycles Observed temperatures are above the frost point on average, but low enough for PSCs to form that contain nitric acid, a component of NO_y[13,29] The NO_y measurements indicate that PSC formation does not generally lead to denitrification The maximum frequency of PSC formation over the continent and inside the vortex occur to the east of the ER-2 flight tracks over the Weddell Sea[30], downwind from the ER-2 flight track The perturbed levels of reactive chlorine initiated by the PSCs cannot be long-lived because of the lack of denitrification In sunlight, nitric acid will gradually photolyse to produce NO_2, and this will reduce ClO levels to near unperturbed values With such intermittent chemical perturbations, this region may contain sufficient recurrent enhancements in ClO to destroy O_3 to the extent indicated in our analysis

Obtaining values for unperturbed O_3 in the Antarctic from other species must carry with it some uncertainty, but the analysis presented here has been conservative In particular, if the value of 300 had been chosen for O_3/NO_y as is indicated by the Southern Hemisphere data, the analysis would have produced somewhat larger O_3 losses The negative O_3 loss 10-15° outside the boundary (Figs 3 and 4c) shows that the choice of 270 for the ratio is conservative Nevertheless, the method is more reliable than the simple 'before and after' comparisons of O_3 profiles which we strongly caution against when analysing losses of O_3 at high latitudes unless the losses are very large, as within the Antarctic ozone hole

We have analysed winter-time high-latitude O_3 changes in a region where O_3 is considered under dynamical control and standard gas-phase photochemistry is extremely slow We have estimated a reference state for O_3 in this region from other conserved trace species and compared this with measured values to determine a minimum loss in ozone In this context we can summarize the important conclusions as follows (1) There is a substantial O_3 loss by mid-August both inside and outside the highly chemically perturbed region, (2) For the first 5° outside the boundary, the average O_3 loss from 23 August to 22 September was at least 15-30% at 17 5 km and 4-25% at 19 km, (3) The O_3 loss found outside the boundary is chemical in origin and did not occur within the highly chemically perturbed region An anthropogenic component cannot be ruled out, (4) For the first 5° inside the boundary and in the layer between 17 5 and 19 km the air observed on 23 August had an O_3 loss of about 40% By 22 September there was an O_3 loss of at least 75%, much larger than the average isentropic temporal decrease of 53% from 23 August to 22 September

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1 Atkinson R J Matthews W A Newman P A & Plumb R A *Nature* **340** 290-294 (1989)
2 Farman J C Gardiner B G & Shanklin J D *Nature* **315**, 207-210 (1985)
3 Stolarski R S *et al Nature* **322**, 808-811 (1986)
4 Hofmann D J Harder J W Rolf S R & Rosen J R *Nature* **326**, 59-62 (1987)
5 Komhyr W D Oltmans S J & Grass R D *J geophys Res* **93**, 5167-5184 (1988)
6 Solomon S Garcia R R & Stordal F *J geophys Res* **90**, 12981-12989 (1985)
7 Garcia R R & Solomon S *J geophys Res* **90**, 3850-3868 (1985)
8 Proffitt M H *et al J geophys Res* **94**, 11437-11448 (1989)
9 Danielsen E F & Houben H *Anthropogene Beeinflussung der Ozonschicht*, 191-242 (DECHEMA Frankfurt am Main 1988)
10 Proffitt M H *et al J geophys Res* (in the press)
11 Tuck A F *J geophys Res* **94**, 11687-11737 (1989)
12 Kelly K K *et al J geophys Res* **94**, 11317-11357 (1989)
13 Fahey D W *et al J geophys Res* (in the press)
14 Galbally I E *et al Q Jl R met Soc* **112**, 775-809 (1986)
15 Ko M K W McElroy M B Weisenstein D K & Sze N D *J geophys Res* **91**, 5395-5404 (1986)
16 Solomon S & Garcia R R *J geophys Res* **89**, 11633-11644 (1984)
17 Russell J M III *et al J geophys Res* **93**, 1718-1736 (1988)
18 Proffitt M H *et al J geophys Res* (in the press)
19 Starr W L & Vedder J F *J geophys Res* **94**, 11449-11463 (1989)
20 Margitan J J *et al J geophys Res* (in the press)
21 Loewenstein M Podolske J R Chan K R & Strahan S E *J geophys Res* **94**, 11589-11598 (1989)
22 Podolske J R Loewentstein M Strahan S E & Chan K R *J geophys Res* (in the press)
23 Brasseu G & Solomon S *Aeronomy of the Middle Atmosphere* 2nd edn (Reidel Dordrecht 1986)
24 Krueger A J *et al The 1987 Airborne Antarctic Ozone Experiment The Nimbus-7 TOMS Data Atlas* (NASA Ref Publ NASA RF 1201 Goddard Space Flight Center 1988)
25 Solomon S *Rev Geophys* **26**, 131-148 (1988)
26 *International Ozone Trends Panel Report 1988* (WMO Report No 18 in the press)
27 UNEP/WMO *Stratospheric Assessment of Stratospheric Ozone 1989* (UNEP/WMO Report in the press)
28 Jones R L *et al J geophys Res* **94**, 11529-11558 (1989)
29 Chan K R Scott S G Bui T P Bowen S W & Day J *J geophys Res* **94**, 11573-11587 (1989)
30 Watterson I G & Tuck A F *J geophys Res* (in the press)

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Re-evaluation of the linkage relationship between chromosome 11p loci and the gene for bipolar affective disorder in the Old Order Amish

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Reanalysis of an Old Order Amish pedigree, to include several new individuals and two changes in clinical status, markedly reduces the probability of linkage between bipolar affective disorder and the Harvey-*ras*-1 oncogene and insulin loci on chromosome 11 This linkage can be excluded using a large lateral extension of the original Amish pedigree

THE affective disorders are a common[1] and heterogeneous group of illnesses that are characterized by abnormalities of mood and cognition, as well as neurovegetative functions such as sleep, appetite and libido[2] Although the aetiology of these disorders is unknown, genetic factors appear to contribute to the development of both bipolar and unipolar depression[3] in many individuals whose families have multiple affected members[4,5] Attempts to delineate genetic loci for the various affective disorders have been hampered by the likely presence of genetic heterogeneity as well as nongenetic forms (phenocopies) of the disorder Several strategies have evolved to circumvent these problems Perhaps the most powerful of these has been the application of genetic linkage techniques to the study of populations that have developed from a restricted gene pool and hence have a more restricted array of alleles predisposing to affective disorder Egeland and co-workers[6] have identified several large pedigrees with a high incidence of affective disorder among the Old Order Amish located in southeastern Pennsylvania The Old Order Amish are a religious sect numbering ~15,000 who descend from some 30 pioneer couples and who have remained genetically isolated, thereby minimizing the introduction of multiple genes responsible for inherited disorders Amish families have large sibships and multiple living generations, making them ideal for genetic studies Alcohol and drug abuse, which often complicate psychiatric diagnoses, are rare among the Amish Bipolar affective disorder, however, occurs with a prevalence rate, characteristic symptom pattern and clinical course that are similar to those in the general North American population[7] The identification and characterization of these pedigrees[6,7] led to the initiation of early genetic linkage studies but no evidence for linkage between various polymorphic serum proteins or blood group antigen loci and affective disorder was found[8] More recently, using a molecular genetic approach, Egeland and colleagues reported evidence supporting the localization of a gene conferring a strong predisposition to bipolar affective disorder linked to two loci located on the short arm of chromosome 11, the Harvey-*ras*-1 oncogene locus (*HRAS*) and the insulin (*INS*) locus[9] Several studies in non-Amish families have excluded linkage between a single locus predisposing to affective disorder and these chromosome 11 markers[10,11]

The current set of analyses extend the original study by adding genotypic data obtained on several members not previously included, by incorporating new clinical data on several recent onsets of affective illness in previously unaffected members of the pedigree, and by extending the number of individuals under study with a large lateral extension of the original pedigree The addition of this new clinical and genetic data identified individuals in the core pedigree who were obligate recombinants with either marker when the diagnostic criteria used in the original study were applied The impact of these new data was to lower the overall lod score and hence weaken the evidence for linkage between an affective disorder locus on 11p15 and the *HRAS* and *INS* loci Also, the large lateral extension of pedigree 110 does not show linkage between these chromosome 11p15 markers and an affective disorder locus When combined with data from the original core pedigree, the hypothesis that a single affective disorder locus is linked to *HRAS* and *INS* in the entire pedigree could be excluded at a distance of 5 centimorgans (cM, a centimorgan is equivalent to 1% recombination between two loci and corresponds to $\sim 1\times 10^6$ base pairs of DNA) for *INS* and 10 cM for *HRAS* We conclude that the evidence for linkage between bipolar affective disorder and the *HRAS* and *INS* genes in the Old Order Amish is not as strong as was initially suggested

The extended and updated pedigree

The original pedigree 110, as previously reported, consisted of 81 subjects and includes the extended families of 6 ill probands 5 with bipolar affective disorder and 1 with schizoaffective disorder, circular type, as well as their first- and second-degree relatives (Fig 1) These patients were assessed by family study methods[12] They and their relatives were interviewed using the Schedule for Affective Disorder and Schizophrenia-Lifetime

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Version (SADS-L)[13]. In addition, all hospital and clinic records were abstracted. The multiple SADS-L interviews and collated records were evaluated by a five-member psychiatric review board who were blind to patient identity and biological relationships. Most patients were diagnosed by the Board at two points in time and the reliability of diagnoses between assessors was high[12]. Nineteen of the 81 subjects in the original pedigree 110 were given diagnoses of major affective disorder according to Research Diagnostic Criteria (RDC)[14]. Fourteen had some form of bipolar disorder and five had unipolar depression. Five persons had minor depressive disorder by RDC and were treated as 'unaffected' in all the linkage analyses. One person who failed to meet RDC diagnostic criteria was diagnosed as having a 'bipolar-type' disorder by consensus of the psychiatric panel. Sixteen of 19 individuals with major affective disorder have been hospitalized at least once, yielding a total of 86 abstracted hospital records for diagnostic review. The availability of these multiple psychiatric evaluations contributed to the reliability of diagnoses in this study. The updated core pedigree includes data on two new unaffected individuals, new genotypes on ten unaffected members not included in the original study, and changes in diagnoses for two individuals owing to onset of illness subsequent to the initial linkage analysis.

The core pedigree of 81 subjects has been extended to include a small left extension closely related to the core pedigree through common great-grandparents (Fig. 1). This left extension consisting of six subjects (two with diagnoses of bipolar affective disorder) has been included in the linkage analysis of the updated core pedigree (Fig. 1 and Table 2). In addition, a large 31-member right extension (Fig. 1) has been studied; it contains eight individuals with a diagnosis of major affective disorder (four with bipolar disorder and four with major depressive disorder). Inspection of Fig. 1 reveals that individual C-4 connects this right extension to the core pedigree. This person and her spouse were psychiatrically well. Both, however, had uncles with RDC diagnoses of bipolar affective disorder (type I) by psychiatric board review. The decision to extend the pedigree through individual C-4, rather than C-3, was based on sibship structure and the segregation of the illness over four generations. Historical and geneological records of the upper generation (generation B, Fig. 1) of this right extension suggests the possibility that affective disorder in this extension may have been introduced by an Amish-Mennonite family whose surname is no longer found among members of the Amish community (Fig. 1). Consequently, for the linkage analysis, the right extension was analysed both separately and combined with the updated core pedigree (Table 2).

As part of the Amish Study, each subject (for whom immortalized cell lines have been established) is visited annually by a staff member. Field work is carried out by staff members who are blind to the genotypes of all subjects. As a result of this longitudinal evaluation, it has been noted that since the initial analysis of the data, the clinical status of two individuals (III-21, IV-20) (Fig. 1) has changed from unaffected to affected.

Defining a diagnostic hierarchy

Relatives of individuals with bipolar affective disorder have been found to have a variety of affective and nonaffective psychiatric syndromes[15]. These include individuals with

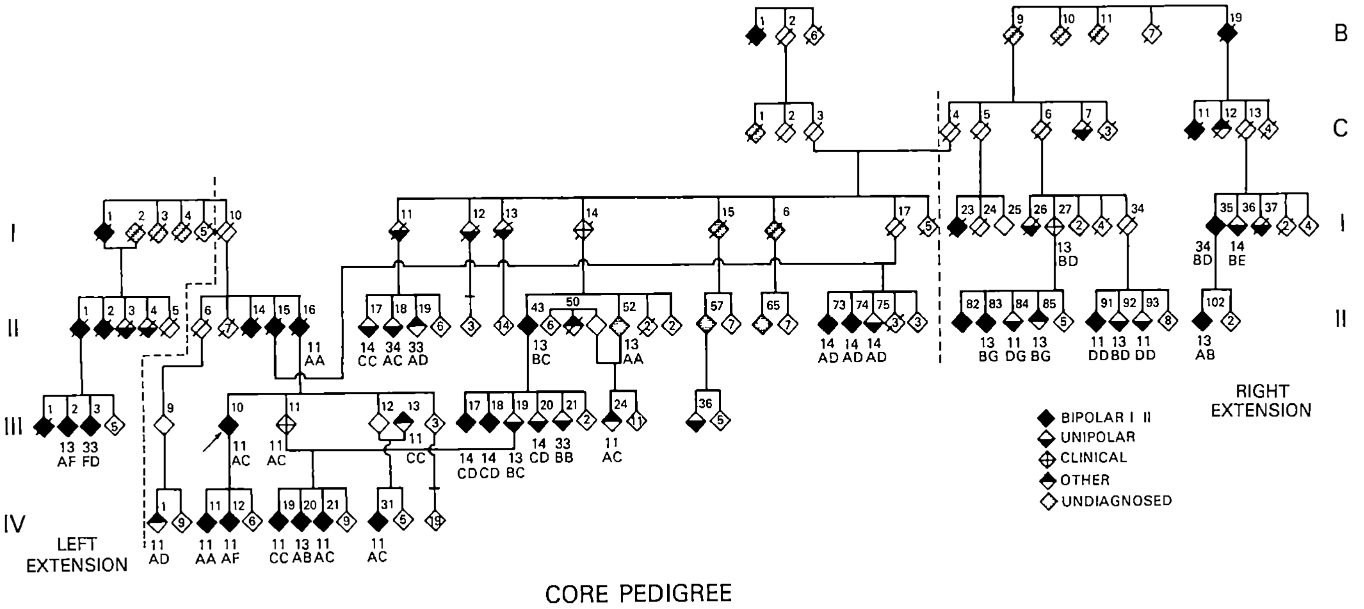

FIG. 1 Pedigree 110 and its extensions. The structure of the 120 member family (pedigree 110) used in this study is shown. The dotted lines distinguish the left and right extensions from the core pedigree. Sex and birth order have been disguised to protect anonymity. Genotypes are not included for the unaffected members to protect those for whom a genetic vulnerability might be indicated. Of those with diagnoses designated 'other', subject III-24 has schizoaffective illness, the rest have minor depressions. Genotypes are designated with numbers, 1–4, for *HRAS*, and with letters, A–G, for *INS*. In several members of the right extension, one laboratory (NIMH) identified another *INS* allele, H, which is 2.4 kb in size, and, therefore, difficult to distinguish from the D allele. In the analyses reported here, the H allele was scored as D, because of the difficulty in resolving the two alleles. The linkage analyses also were carried out using the H allele resulting in somewhat more negative lod scores, but changing neither the basic conclusions nor the size of the exclusionary range (data not shown). Genotypes were determined independently in two laboratories, NIMH and St Louis. In the initial analysis several discrepancies in genotypings were obtained and were resolved by reanalysis in both laboratories except for one individual (III-20), and a new DNA sample will be needed to resolve this discrepancy. The St Louis genotypings were carried out as previously described[9]. In the NIMH protocol, genomic DNA was digested with the appropriate endonuclease (*SstI* for *HRAS* or *PvuII* for *INS*, BRL) and 10 μg was electrophoretically separated in either 0.8% agarose and Oncor buffer (*HRAS*), 1.0% agarose and TBE (insulin alleles A, B, C, E, F), or 1.0% agarose and Oncor buffer (insulin alleles D, G). The DNA was transferred by vacuum to nylon membranes (Oncor). Probes for *HRAS* (pEJ 6.6) and *INS* (phins 310) were obtained from American Type Culture Collection (Rockville) and labelled by the random primer method[22] using [α-^{32}P]dCTP (Amersham). Hybridization was carried out at 45 °C overnight using the appropriate probe and Hybrisol I (Oncor) with poly-A RNA (10 μg ml^{-1}) and denatured *E. coli* DNA (100 μg ml^{-1}) added. Membranes were washed three times in 0.1% SDS, 0.1×SSC at room temperature for 15 min, followed by one wash at 52 °C for 1 h. Autoradiography was performed using Kodak XAR film and enhancing screens. Films were read independently by two people unaware of the diagnoses.

schizoaffective disorder, unipolar depressive disorder, cyclothymia, dysthymia or depressive neurosis and various personality disorders with and without affective symptoms As pedigree 110 and its extensions include a number of individuals with these psychiatric diagnoses (Fig 1), which may (or may not) be genetically related to bipolar affective disorder, a diagnostic hierarchy was developed to include such individuals in the linkage analysis This hierarchical system has two dimensions The first measures severity of affective symptoms and has four categories (Table 3) The other dimension includes milder syndromes in the affected phenotype by allowing for both a criteria-based system such as the RDC (as was used in the initial study, ref 9) as well as the clinical impressions of psychiatrists on the review board The diagnostic hierarchy used in the present study (Table 3 legend) was developed by members of the psychiatric review board who were unaware of the genotypes of any of the members of the pedigree

Genotyping and linkage analysis

Genotyping was performed independently in two laboratories (NIMH and St Louis) Genomic DNA was extracted from lymphoblastoid cell lines obtained from the Coriell Institute for Medical Research[16] for 120 members of Amish pedigree 110 (Fig 1 and Table 2) In one laboratory (St Louis), DNA for some family members was extracted directly from blood The methodology for DNA extraction and Southern analysis differed slightly between the two laboratories (see legend Fig 1) A consensus was obtained on all typings, however, with the exception of one individual (III-20) Resolution of this single discrepant genotype will require obtaining a new DNA sample on individual III-20 as each laboratory was able to confirm their typing on several occasions from their original source of DNA, indicating that an error had occurred in the handling and (or) storage of lymphocytes for this subject Nevertheless, the linkage analyses were performed twice using each genotyping for this subject without any effect on the lod scores (data not shown)

TABLE 1 Age-specific penetrances used for multipoint linkage analyses

Age	Liability class	Probability of disease AA	Aa	aa
0–14	1	0 0001	0 0001	0
15–19	2	0 18	0 18	0 0001
20–24	3	0 42	0 42	0 001
25–29	4	0 73	0 73	0 001
⩾30	5	0 85	0 85	0 001

Values represent age- and genotype-specific penetrances of the Major Affective Disorder (MAD) phenotype AA represents an individual homozygous for the disease alleles, Aa represents heterozygotes, and aa represents individuals genotypically normal at the MAD locus

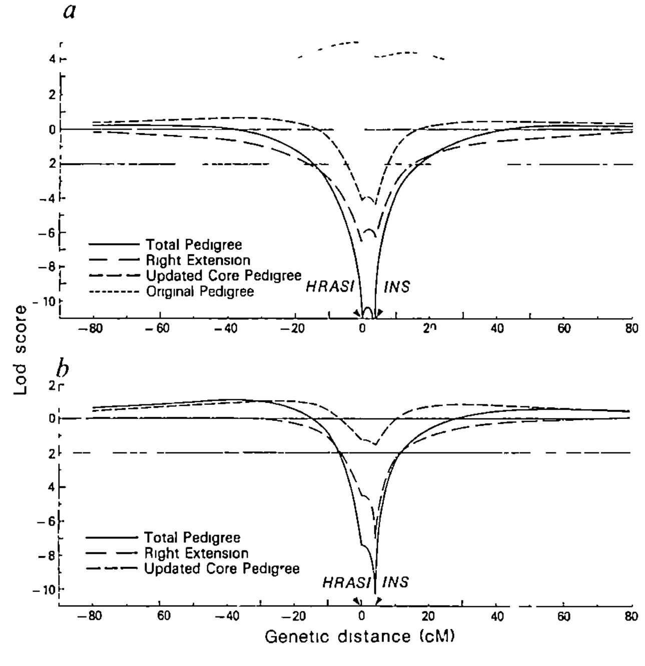

FIG 2 Multipoint linkage analysis using *A*, the original diagnostic criteria and *b*, the diagnostic system which includes broader diagnostic criteria to define the affected phenotype System A is equivalent to system number 3 (RDC) from Table 3, and includes affected individuals with bipolar disorder, major affective disorder and schizoaffective disorder, but not minor depression System *B* is identical to system 2 using clinical criteria from Table 2 It includes as affected those with bipolar, schizoaffective or recurrent unipolar major affective disorders, but excludes those with minor depression or only a single episode of major depression For each diagnostic system, the data has been analysed for the updated core pedigree, the right extension and the total pedigree Three-point analyses to determine the most likely location of the locus for bipolar affective disorder in relation to *HRAS* and *INS* were performed using the LINKMAP subroutine of LINKAGE version 4 8 (ref 20) For these analyses, the distance between *HRAS* and *INS* was held constant at 3 8 cM (ref 23) Five liability classes were defined (see Table 1) The recombination distance between *HRAS* and *INS* was examined in each portion of the pedigree and found to be consistent with other estimates[23]

Four *HRAS* alleles (about 5 2 kilobases (kb) long (1), ~5 7 kb (2), ~6 2 kb (3), ~6 8 kb (4)) and four *INS* alleles (about 760 bp (A), ~800 bp (B), ~830 bp (C), ~2 35 kb (D)) were delineated as described in the original study[9] Three additional *INS* alleles (~650 bp (E), ~820 bp (F), ~2 5 kb (G)) were also observed, primarily in the extensions of the core pedigree The microheterogeneity observed at the *INS* locus is consistent with other population studies of this gene[17]

Analyses of the cosegregation of alleles at the *HRAS* and *INS* loci with the bipolar affective disorder phenotype were performed using the computer programs LIPED[18,19] and LINKAGE[20] (see Table 2 legend) Analysis of linkage between affective disorder and each marker was done for the original core pedigree, the updated core pedigree, the right extension and the complete pedigree Five liability classes were defined and the age-specific penetrances for the multipoint linkage analysis are shown in Table 1

Linkage in the updated core pedigree

In the course of our study we (J K , E G , S B , A G , R L , G C and S P, unpublished data) independently reanalysed the original core pedigree with the affection status and diagnoses used in the initial study, but with the genotypes from the NIMH laboratory, and identical lod scores were obtained The genotypes of 2 new unaffected family members (II-20, 21) added into the core pedigree had only a minor effect on the lod scores The addition of genotypes for 10 unaffected individuals, however, who were incompletely genotyped in the original analysis (data from only one of the two marker loci were available), resulted in a significant change in the lod scores for both *HRAS* and *INS* The maximum lod score for *HRAS* decreased from 4 08 at $\Theta = 0$ to 2 46 at $\Theta = 0\ 05$, whereas that for *INS* increased from 2 63 to 3 38 at $\Theta = 0$ (Table 2)

The most dramatic effect on the lod scores resulted from the addition of updated diagnostic information on two family members who became ill after the original study, one (IV-20) developed a manic psychosis (bipolar affective disorder, type I) and the other (III-21) suffered a severe depression with catatonic features (major depressive disorder) Together, these changes lowered the interpolated maximum lod scores to 1 03 at $\Theta = 0\ 17$ and 1 75 at $\Theta = 0\ 14$ for *HRAS* and *INS*, respectively (Table 2) The addition of the left extension to the core pedigree (updated core) further reduced the lod scores to a maximum of 0 70 at $\Theta = 0\ 20$ and 1 29 at $\Theta = 0\ 20$ for *HRAS* and *INS*, respectively (Table 2)

TABLE 2 Lod scores resulting from sequential expansion of the core pedigree

Information added	Recombination frequency (Θ) 0.00	0.001	0.05	0.10	0.20	0.30
Locus *HRAS*						
1 Original core (*N*=81)	4.08	4.08	3.91	3.63	2.85	1.81
2 1+new genotypes (*N*=83)	2.36	2.37	2.46	2.39	1.92	1.25
3 2+updated DX	−1.75	−1.53	0.44	0.91	1.01	0.63
4 Updated core (3+left ext., *N*=89)	−3.22	−2.97	−0.34	0.36	0.70	0.46
5 Right ext (*N*=31)	−6.15	−5.83	−2.95	−1.93	−0.85	−0.32
6 Total (4+5, *N*=120)	−9.31	−8.93	−3.97	−2.21	−0.65	−0.21
Locus *INS*						
1 Original core (*N*=81)	2.63	2.63	2.50	2.31	1.85	1.50
2 1+new genotypes (*N*=83)	3.38	3.38	3.24	3.02	2.43	2.23
3 2+updated DX	−0.19	0.05	1.45	1.69	1.62	1.21
4 Updated core (3+left ext., *N*=89)	−1.90	−1.64	0.41	0.99	1.29	1.07
5 Right ext (*N*=31)	−4.81	−4.54	−2.46	−1.73	−0.84	−0.35
6 Total (4+5, *N*=120)	−7.75	−6.71	−2.40	−1.07	0.19	0.55

The original core pedigree is identical in membership, genotypes and diagnoses to that reported originally[9]. Each subsequent analysis includes the information in the previous one plus the additional data as indicated. The new genotypes include 10 *HRAS* and *INS* genotypes which were not available at the time of the original study. The updated diagnoses refer to two individuals who had an onset of major affective disorder subsequent to the initial analysis (IV-20, bipolar I disorder, II-21, major depressive disorder). Analyses of the cosegregation of alleles at the *HRAS* and *INS* loci with the bipolar affective disorder phenotype were performed using the computer programs LIPED[18,19] and LINKAGE[20] on a COMPAQ Deskpro 386 microcomputer and a VAX 8800. All pairwise analyses (*HRAS* × *INS*, *HRAS* × affective disorder, *INS* × affective disorder) used LIPED. Analyses were performed assuming an autosomal dominant transmission model. For the two-point analyses, the probability of exhibiting the affected phenotype for individuals who inherited the predisposition allele for bipolar affective disorder was modelled as a linear function of age with a penetrance of 0 at age 15 and a maximum penetrance of 85% reached at 35 years of age. The maximum penetrance for the nonsusceptible genotype was 0.1%, reached at 35 years of age. The allele frequency of the disease-predisposition allele was taken as 0.021 for all analyses. The *SstI HRAS* allele frequencies were 0.30, 0.30, 0.10, and 0.30 for alleles 1, 2, 3, and 4, respectively. Genotypes at the *INS* locus were reduced to a four-allele system to maximize efficiency of computing time. The latter involves no loss of information in the analyses. All alleles were considered as equally likely.

Linkage in the right extension

When analysed alone, the lod scores for linkage between either the *HRAS* or *INS* loci and bipolar affective disorder in the right extension were strongly negative (Table 2); linkage can be excluded for $\Theta \leq 0.05$. Similarly, when the right extension and core are considered together, linkage can be excluded for $\Theta \leq 0.10$ for *HRAS* and $\Theta \leq 0.05$ for *INS*. The lod scores at $\Theta = 0$ represent odds of 10^{10} and 10^{8} against close linkage between a disease locus and the *HRAS* and *INS* loci, respectively.

Alternative diagnostic criteria

Linkage analyses were done using alternative diagnostic criteria (clinical consensus diagnoses) as outlined in Table 3 on the core pedigree. The strongest evidence for linkage was obtained when all individuals with mania, hypomania or recurrent major depression were considered affected and when clinical, as opposed to RDC, diagnoses are used (maximum lod score for *INS* of 2.88 at $\Theta = 0$) (Table 3). Inclusion of individuals with minor depression decreased the lod scores for both *HRAS* and *INS*.

Multipoint linkage analysis

Illustrated in Fig. 2*a* are the results of the multipoint linkage analysis between *HRAS*, *INS* and a locus for bipolar affective disorder using RDC diagnostic criteria. Linkage was excluded in the interval between *HRAS* and *INS*, as well as to within 5 cM on either side of each of these markers for the updated core pedigree. Both the right extension alone and the total pedigree gave exclusions to within 15 cM on either side of the *HRAS*-*INS* interval.

Figure 2*b* illustrates the multipoint linkage results when a clinical (as opposed to RDC) diagnostic system is used to define the affected phenotype including mania, hypomania and recurrent major depressive disorder. Using this diagnostic system, linkage could not be excluded in the core pedigree alone; the maximum lod score was $\sim$1.0 at 26 cM distal to *HRAS*. Linkage could be excluded in the right extension and in the total pedigree within the interval between *HRAS* and *INS* and to within 7 cM on either side of the markers.

Discussion

The initial linkage analyses of pedigree 110 reported by Egeland *et al*[9] provided strong evidence for a locus conferring a predisposition to manic-depressive illness on chromosome 11p15. These investigators reported a lod score of 4.08 at $\Theta = 0$ in the two-point analyses between *HRAS* and bipolar affective disorder. The multipoint linkage analysis using both markers, *HRAS* and *INS*, yielded a lod score of $\sim$5 (odds of 10^5 to 1 in favour of linkage). In our reanalysis, the recent onset of affective disorder in 2 members of the pedigree and the addition of genotypic data on six individuals in a newly included sibship, and on ten previously incompletely genotyped members, markedly reduces the statistical probability of linkage in both the two-point and multipoint analysis (Table 2 and Fig. 2).

Linkage between bipolar affective disorder and the *HRAS* and *INS* loci can also be excluded in the large lateral extension of pedigree 110. This observation can be interpreted in at least two ways: that there is genetic heterogeneity among the Old Order Amish, and/or that, if it is assumed that the illness in the original core and this extension is genetically homogeneous, there is strong evidence against linkage of a susceptibility locus for bipolar affective disorder to this region of 11p15. Although the Old Order Amish represent a genetic isolate, it has been suggested (J. A. Egeland, personal communication) that multiple genes may be responsible for affective disorder within the Amish population as several progenitor couples had mental illness among their first and second generation descendants. The analysis, therefore, of a large portion of the core pedigree may be complicated by the presence of two different genes responsible for affective illness.

What then is the evidence that bipolar affective disorder is linked to the *HRAS* and *INS* loci in the Old Order Amish? The hypothesis that has been tested so far is that the inheritance of specific alleles at a locus on chromosome 11p15 linked to *HRAS* and *INS* is a necessary condition for the occurrence of affective disorder in the members of pedigree 110. This hypothesis has been rejected in the analysis of the extended pedigree. Alternative hypotheses consistent with the expanded data set and analysis include the possibility that linkage to 11p15 markers in the

TABLE 3 Lod scores in core pedigree using alternate diagnostic criteria

Diagnoses included	Recombination frequency (Θ) 0.00	0.05	0.10	0.15	0.20	0.25	0.30
Locus *HRAS*							
1 Mania or hypomania only							
RDC	0.29	0.65	0.78	0.81	0.78	0.69	0.57
Clinical	0.53	0.59	0.61	0.60	0.56	0.49	0.40
2 1+recurrent MDD							
RDC	0.69	1.17	1.33	1.32	1.19	0.99	0.73
Clinical	1.34	1.49	1.49	1.39	1.21	0.97	0.69
3 2+MDD single episode							
RDC	−1.02	0.71	1.06	1.15	1.08	0.92	0.68
Clinical	−0.59	1.02	1.23	1.22	1.10	0.90	0.65
4 3+minor dep							
RDC	−4.23	−1.30	−0.53	−0.13	0.08	0.17	0.17
Clinical	−3.66	−0.99	−0.37	−0.05	0.10	0.16	0.14
Locus *INS*							
1 Mania or hypomania only							
RDC	1.03	1.09	1.08	1.02	0.92	0.78	0.63
Clinical	1.28	1.19	1.07	0.94	0.81	0.68	0.54
2 1+recurrent MDD							
RDC	2.22	2.30	2.25	2.11	1.91	1.65	1.35
Clinical	2.88	2.80	2.63	2.39	2.10	1.77	1.42
3 2+MDD single episode							
RDC	0.36	1.82	1.98	1.94	1.80	1.58	1.30
Clinical	0.94	2.33	2.36	2.22	1.99	1.70	1.37
4 3+minor dep							
RDC	−3.35	−0.14	0.50	0.79	0.90	0.90	0.80
Clinical	−2.76	0.36	0.88	1.07	1.10	1.02	0.87

The effect of different diagnostic criteria on the lod score analysis in the updated core pedigree. These data were calculated from a version of the updated core pedigree that lacks the small left extension, but includes all other updated information. See the legend of Table 2 for details of the two-point linkage analyses. Various diagnostic criteria (RDC, clinical) were used by the psychiatric review board who were blind to genotypes or the effects of diagnoses on the lod scores. The hierarchy is devised along two dimensions: one is based on the degree of relatedness of a given diagnosis to bipolar affective disorder, the other is based on whether the diagnoses were made by RDC criteria or by the clinical impressions of the review board. For each diagnostic system, lod scores are reported for both RDC and clinical diagnoses. The first diagnostic system includes as affected only those individuals who have had mania or hypomania. The next adds those individuals who have had multiple episodes of major depressive disorder (MDD), followed by a system that includes those who have had only a single episode of MDD. The final diagnostic system includes any individual who has had a diagnosis of minor depressive disorder, which requires fewer symptoms than major depression.

core is due merely to chance. Many other DNA markers have been tested in the core pedigree but no evidence for linkage has yet been found[21]. Testing of markers spanning the remainder of the genome will lead to the identification of the site of this locus, if a single major locus hypothesis is correct. Even a complete systematic evaluation of pedigree 110, however, using highly polymorphic markers spaced very closely and covering the entire genome may not resolve the issue. If genes at more than one locus can independently lead to genetic susceptibility to affective disorder in this pedigree, then conventional linkage analysis over the entire pedigree will continue to be problematic. It may, therefore, be impossible to generate a strong positive lod score over the entire pedigree for linkage of a single major affective disorder locus to any marker.

Another important consideration is the possibility that non-genetic factors may contribute to the aetiology of affective disorder in pedigree 110. Any individual in whom illness was significantly mediated by environmental rather than genetic factors has an even chance of appearing as a recombinant in a linkage analysis. If the frequency of such phenocopies is low, then their impact on statistical tests of genetic hypotheses may not be great, unless they happen to occur in a critical part of the pedigree. As the frequency of such individuals increases, however, their impact on the tests of genetic hypotheses may become increasingly significant. This may be particularly problematic when diagnostic categories are broad and the population frequency of individuals considered affected becomes correspondingly high. The use of narrower or alternative diagnostic criteria may reduce the frequency of phenocopies and hence the probability of observing chance recombination. Until a systematic analysis of the entire genome has been completed, it will not be possible to determine the extent to which these problems confound the identification of loci which contribute to the aetiology of affective disorder in pedigree 110.

Our analyses highlight many of the problems that can be anticipated in genetic linkage studies of common and complex neuropsychiatric disorders. Foremost among these are the probable presence of genetic heterogeneity even in relatively isolated populations and the presence of phenocopies, especially in large pedigrees such as in the Amish. Longitudinal follow up and reanalysis of the data incorporating unaffected members of the pedigree who subsequently become ill are essential, given the variable age of onset for psychiatric disorders. Molecular genetic studies of psychiatric disorders will continue to encounter these problems and efforts to reduce their impact will be critical. ☐

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1 Reiger D A *et al Arch Gen Psychiat* **45,** 977-986 (1988)
2 Lehmann H in *Comprehensive Textbook of Psychiatry IV* (eds Kaplan H I & Saddock B J) 786-811 (Williams and Wilkins Baltimore, 1985)
3 Perris C *Acta Psychiat Neurol Scand Suppl* **42,** suppl 194 (1966)
4 Nurnberger J I & Gershon E S in *Neurobiology of Mood Disorders* (eds Post R M & Ballenger J C) 76-101 (Williams and Wilkins Baltimore 1984)
5 Goldin L R Gershon E S Targum S D Sparkes R S & McGinnis M *Am J hum Genet* **35,** 274-287 (1983)
6 Egeland J A & Hostetter A M *Am J Psychiat* **140,** 56-61 (1983)
7 Egeland J A Hostetter A M & Eshleman S K *Am J Psychiat* **140,** 67-71 (1983)
8 Kidd K K *et al Am J Psychiat* **141,** 1042-1048 (1984)
9 Egeland J A *et al Nature* **325,** 783-787 (1987)
10 Hodgkinson S *et al Nature* **325,** 805-806 (1987)
11 Detera Wadleigh S *et al Nature* **325,** 806-808 (1987)
12 Hostetter A M Egeland J A & Endicott J *Am J Psychiat* **140,** 62-66 (1983)
13 Endicott J & Spitzer R L *Arch Gen Psychiat* **35,** 837-844 (1978)
14 Spitzer R L Endicott J & Robins E *Arch Gen Psychiat* **35,** 773-782 (1978)

15 Nurnberger J I & Gershon E S in *Handbook of Affective Disorders* (ed Paykel E S) 126–145 (Guilford New York 1982)
16 Egeland J A in *Catalog of Cell Lines NIH Publication 87 2011* 298-306 (National Institutes of Health Washington D C 1987)
17 Rotwein P Yokoyama S Didier D K & Chirgwin J M *Am J hum Genet* **39,** 291–299 (1986)
18 Ott J *Am J hum Genet* **28,** 528–529 (1976)
19 Hodge S E Morton L A Tideman S Kidd K K & Spence M A *Am J hum Genet.* **31,** 761–762 (1979)
20 Lathrop G M Lalouel J M Julier C & Ott J *Proc natn Acad Sci USA* **81,** 3443–3446 (1984)
21 Kidd J R *et al J Psychiat Res* **21,** 577-580 (1987)
22 Feinberg A P & Vogelstein B *Analyt. Biochem* **132,** 6-13 (1983)
23 White R *et al Nature* **313,** 101-105 (1985)

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Scanning from an independently specified branch point defines the 3′ splice site of mammalian introns

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During pre-messenger RNA splicing the lariat branch point in mammalian introns is specified independently of the 3′ splice site by the sequence surrounding the branch point and by an adjacent downstream polypyrimidine tract The 3′ splice site is dispensable for spliceosome assembly and cleavage at the 5′ splice.site, and is itself determined by a scanning process that recognizes the first AG located 3′ of the branch point/polypyrimidine tract, irrespective of distance or sequence environment

THE splicing of pre-messenger RNA involves the accurate and efficient removal of introns to produce mature mRNA which is a fraction of the size of the primary transcript The splicing reaction occurs by means of two consecutive transesterification steps[1] The RNA is first cleaved at the 5′ splice site, and a 2′-5′ phosphodiester bond is formed between the 5′ end of the intron and the 2′-OH group of a specific 'branch point' nucleotide within the intron In the second step, the 3′ splice site is cleaved, releasing the branched 'lariat' form of the intron, and the two exons are ligated together The *cis* information for splicing is restricted to limited regions of the transcript which interact with multiple RNA and protein factors to form a 'spliceosome' complex, in which the splicing reaction takes place[1-4] The GU and AG dinucleotides at the 5′ and 3′ intron boundaries are nearly invariant in higher eukaryotes, as is the A at the branch point[1,5-7] In addition, there is a consensus sequence around the 5′ splice site, $^{C}_{A}$AG-GURAGU (refs 6, 7), which is involved with base pairing to U1 small nuclear RNA (snRNA)[8], a stretch of pyrimidines typically located immediately upstream of the 3′ splice site and a weak mammalian consensus branch-point sequence, YNYURAY, which binds to U2 small nuclear ribonucleoproteins[9] (snRNPs) potentially by base pairing with U2 snRNA[5,10,11] In yeast there is an invariant branch point sequence, UACUA$\underline{\text{A}}$C, which base pairs with U2 snRNA (ref 10) Increasing the base pairing potential between mammalian branch points and U2 snRNA improves their efficiency[12-15] Nevertheless, many mammalian branch points bear little resemblance to the consensus, several lines of evidence, however, have led to the belief that the mammalian branch point is specified primarily by its proximity to the 3′ splice site, possibly by a device that measures distance from the AG dinucleotide at the 3′ intron boundary[5,16,1] First, most mammalian branch points have been mapped within 18–40 nucleotides of the 3′ splice site[5,16,18] Second, mutation of branch points can lead to activation of cryptic sites within the same distance[16,17] Third, some introns lack A residues within 18–40 nucleotides of the 3′ splice site, and use U or C residues instead, despite the clear preference for A residues[19] In addition, it has been shown that, to variable extents, mutation of the 3′ splice site AG can inhibit the first step in the splicing reaction, even though the 3′ splice site is not itself cleaved until the second step[18,20-23]

Although the definition of the 5′-splice-site consensus sequence and its recognition by U1 RNA is well characterized[8,24-26] the mechanism of recognition, relative importance and interactions of the 3′ splice site elements, including the branch point, polypyrimidine tract and the 3′ splice site AG, are poorly understood The recent reports of branch points at distances of >140 nucleotides from the 3′ splice site[27-29], clearly indicates that simple proximity to the 3′ splice site cannot be an obligatory determinant of branch-point location

We have now studied the mechanism of branch-point selection in the intron between the mutually exclusive exons 2 and 3 of the rat α-tropomyosin gene (*TM*) This intron has a branch point that lies 172 nucleotides upstream of the 3′ splice site (Fig 1*a*) The proximity of this branch point to the 5′ splice site of exon 2 enforces mutually exclusive behaviour, because splicing factors are unable to bind productively to the two elements and form an active spliceosome[27,28] Using this *TM* intron and the first intron of the human β-globin gene, we show that the branch point is specified independently of the 3′ splice site, by its immediate sequence context and by proximity to an adjacent polypyrimidine tract which may be distant from the 3′ splice site AG Efficient spliceosome formation, cleavage at the 5′ splice site and lariat formation require the polypyrimidine tract and branch-point sequence, but can occur in the complete absence of the 3′ splice site and exon The 3′ splice site itself is only recognized in the second step of splicing, and is in fact determined with reference to the branch point by a distance-independent scanning process for the first AG dinucleotide downstream of the branch point/polypyrimidine tract an extensive stretch of pyrimidines was still present 50–60 nucleotides downstream of the branch point (positions 162–211, Fig 1*a*) Further deletions bringing the branch-point sequence adjacent to this polypyrimidine tract restored splicing activity (Fig 2*a*, Δ44–157) This shows that a functional polypyrimidine tract does not have strict sequence specificity, and that to be active it requires an adjacent acceptable branch-point sequence

Branch-point sequence context

Branch point formation can occur at sequences that are highly divergent from the consensus sequence[16,17] Nevertheless, A residues, such as those at positions 156, 161 and 186 in the *TM* intron (Fig 1*a*), which have a suitable downstream pyrimidine tract, are not used as branch points under any conditions tested To test the role of sequence context in preventing the use of such nucleotides as branch points, the sequence around the A residue 28 nucleotides upstream of the 3′ splice site was converted to the strong branch-point consensus UACUAAC (ref 13) This mutation resulted in splicing of exon 2 to exon 3 in transcripts in which the normal branch point is too close to the 5′ splice site to be involved in branch formation (Fig 2*b*, UACUAAC 181) Thus the pyrimidine environment downstream of position 186 is sufficient to support splicing, given a suitable adjacent branch-point sequence The normal sequence at this position, CCCCCAC, is not an acceptable branch-point sequence, despite its having only two mismatches from the YNYURAY consensus The replacement of the five purines between the UACUAAC and the 3′ splice site with pyrimidines, thereby creating a 25-nucleotide continuous polypyrimidine tract, dramatically increased the efficiency of splicing and of 60S complex formation (UACUAAC 181Y) Thus, the sequence context of the branch point, and an adjacent polypyrimidine tract are involved in branch-point specification, and in determining the efficiency of the splicing reaction

Scanning for the 3′ splice site

Given that the specification of the branch point is independent of the 3′ splice site AG, the question arises as to the specification of the 3′ splice site itself Inspection of the *TM* intron shows that the 3′ splice site is the first AG dinucleotide downstream of the branch point and polypyrimidine tract (Fig 1*a*), as it is in other introns[5,29] Thus, the 3′ splice site could simply be recognized by 5′-to-3′ scanning for an AG, as originally suggested[6,34] To test this possibility, several mutants were constructed in which AG dinucleotides were inserted between the branch point and the 3′ splice site (Fig 3) In all cases, the intervening AG was used exclusively as the 3′ splice site The nature of the nucleotide preceding the AG, however, had a profound effect on the efficiency of the second step in splicing, CAG, UAG and AAG trinucleotides were used efficiently as 3′ splice sites (constructs ppR, p23Bg, p23Af) By contrast, GAG was inefficiently cleaved, although it was efficiently recognized, because downstream AG dinucleotides, including the normal 3′ splice site, were not used Moreover, a CAG-to-GAG mutation (pGAG, Fig 3) severely reduced the rate, but not the position, of cleavage at the normal 3′ splice site These results are compatible with the absence of GAG trinucleotides at natural 3′ splice sites[1,5,7] Deletion of the normal 3′ splice site AG (pΔAG) resulted in the exclusive and efficient use of the first AG in the 3′ exon, 178 nucleotides downstream of the branch point The distance between the branch point and the AG in these transcripts (75–178 nucleotides) had no effect on the efficiency of the second step of splicing (compare p23Bg and pΔAG) All the AG dinucleotides used in the above transcripts had a pyrimidine-rich environment immediately upstream, which could have been involved in specification of the 3′ splice site This is unlikely because conversion of the 30 nucleotides immediately upstream of the 3′ splice site in construct pGC+DX from 70% pyrimidine to 70% purine had no effect on either step in splicing (pR2, Fig 3) This confirms that the polypyrimidine tract is not obligatorily associated with the 3′ splice site Rather, it seems that the 3′ splice site is recognized by a simple scanning process for the first AG downstream of the branch point This is in agreement with the behaviour of some splicing mutants that use AG dinucleotides upstream of the normal 3′ splice site[18,34-37] One possible caveat is that the AG may need to be beyond a minimal distance (>18 nucleotides) from the branch point[5,34,38], presumably because of the steric constraints of factors bound at the branch point and polypyrimidine tract Also, in some cases more than one of a series of closely spaced AG dinucleotides is used as a 3′ splice site (ref 39, and R Reed, personal communication) Nevertheless, in these cases it is the upstream site that is predominantly used[39]

In a preliminary investigation of the nature of the scanning process, we tested whether stable secondary structure between the polypyrimidine tract and the 3′ splice site interferes with the recognition of the 3′ splice site Insertion of a 48-nucleotide palindromic element downstream of the polypyrimidine tract resulted in a block in the second step of splicing (p23HP, Fig 3) The presence of stable secondary structure was confirmed by the resistance to digestion with RNAse H in the presence of

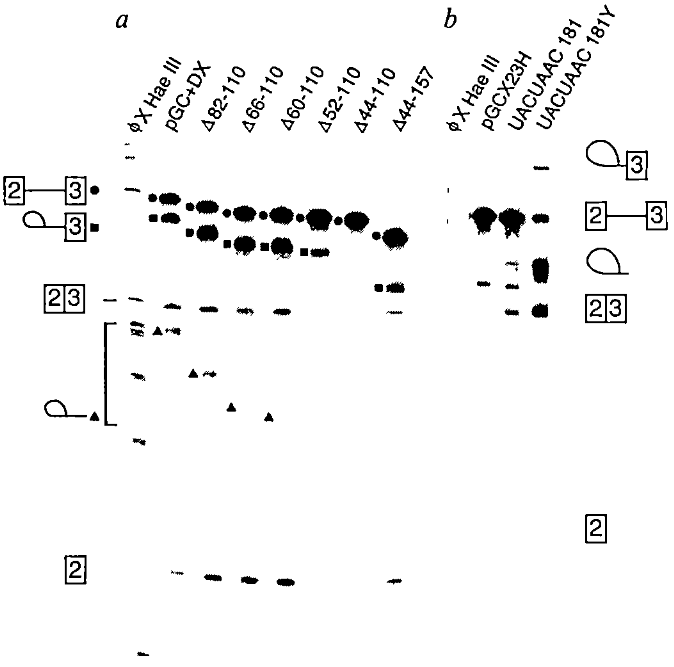

FIG 2 Branch-point specification by adjacent polypyrimidine tract and by sequence context *a*, Effect of polypyrimidine-tract deletions Templates containing specific intron deletions were constructed by deleting between the *AccI* site at position 110 (see Fig 1*a*) and *AccI* sites introduced by oligonucleotide directed mutagenesis with polymerase chain reaction (PCR) amplification, at the indicated positions The structure of mutagenized and amplified DNA was verified by sequencing The templates all contained the spacer element at the 5′ end of the intron, as in plasmid pGC+DX Splicing reactions were carried out and analysed as in Fig 1*b* Two-hour splicing reactions are shown *b*, Branch-point sequence context is important for splicing Constructs containing the native non-splicing intron with no spacer element at the 5′ end (pGCX23H), and with the sequence context of the A residue at position 186 changed from CCCCCAC to UACUAAC (UACUAAC 181 and UACUAAC 181Y) were used as templates for transcription UACUAAC 181Y also had all purines between the 3′ splice site and UACUAAC sequence converted to pyrimidines (U at positions 192, 199 and 205, C at positions 194, 202 and 206) The products of 2-h splicing reactions are shown The only band appearing in the plasmid pGCX23H lane corresponds to a block to endogenous exonuclease activity by factors bound at the branch point[27] The identities of lariat bands produced by the UACUAAC-element-containing transcripts were verified by debranching reactions

oligonucleotides complementary to one side of the stem (data not shown) Transcripts containing either half of the palindromic sequence alone were, by contrast, efficiently spliced (data not shown) Thus, secondary structure seems to be capable of blocking the scanning process that locates the 3′ splice site This result precluded our testing the requirement for a continuous phosphodiester backbone by a *trans* splicing experiment[40], because the complementary elements necessary to bring the two half-transcripts together would themselves block the scanning process In yeast introns, potential secondary structure-forming elements were found to inhibit the second step in splicing when adjacent to the branch point, but not when further downstream[41] Irrespective of secondary structure, however, insertions between yeast branch points and 3′ splice sites decrease the efficiency of the second step in splicing[41,42], indicating some difference in the mechanism of 3′ splice site recognition in yeast and mammals

Generality of specification mechanisms

To test the generality of our findings with the α-TM intron we made several constructs by using the first intron of the human β-globin gene A 120-nucleotide spacer element derived from the *α-TM* intron, chosen because it lacks any cryptic branch points or 3′ splice sites[27], was inserted between the globin gene polypyrimidine tract and 3′ splice site (Fig 4, BP BAM) This intron was still spliced, albeit with reduced efficiency The correct globin splice sites and branch point, now 155 nucleotides upstream of the AG, were used, showing that the branch point need not be in proximity to the 3′ splice site The reduced splicing efficiency could be due to the increased distance of the branch

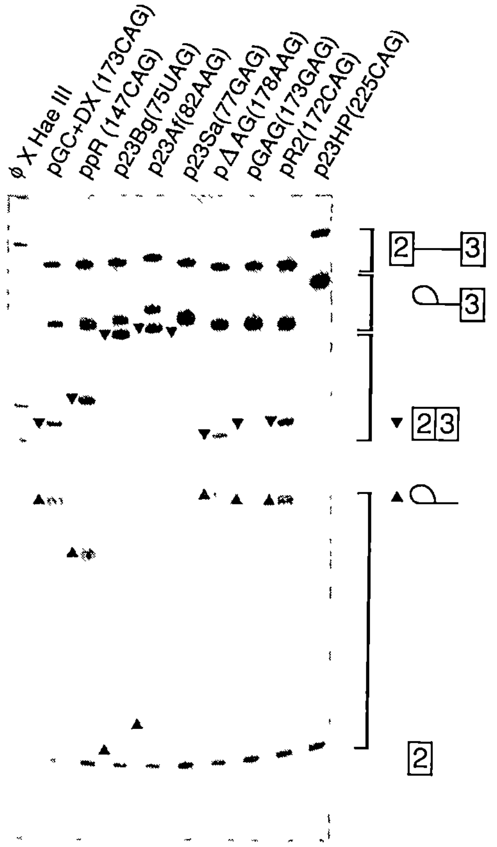

FIG 3 The 3′ splice site is specified as the first AG 3′ of the polypyrimidine tract Constructs with AG dinucleotides inserted between the polypyrimidine tract and 3′ splice site of the pGC+DX template were prepared Plasmid ppR had intron positions 186–213 changed to AGGCGCGTACGGGCACGGAAAACGAACAG Plasmids p23Bg, p23Sa and p23Af had the linker sequences AGATCT, CGAGCTCG and GCACTTAAGTGC inserted into the *Acc*I site at position 110, respectively pΔAG had the AG of the 3′ splice site deleted and, in addition, positions 181–187 changed to UACUAAC The UACUAAC was not used as a branch point in this transcript because of competition from the native branch point Transcripts with an AG-to-GG mutation in the 3′ splice site and no UACUAAC sequence have given identical results Plasmid pGAG had a C-to-G mutation at position 211 Plasmid pR2 had the A residue at position 186 deleted, and positions 187–213 changed to GACGCGTACGGCACGGAAAACGAACAG Plasmids ppR pΔAG, pGAG and pR2 were all cloned by oligonucleotide-directed mutagenesis with PCR amplification as in Fig 2 Plasmid p23HP had the 48-nt palindromic sequence, GGG(CGG)$_6$GAATTC(CCG)$_6$CCC cloned into the *Acc*I site at position 110 Each lane shows the products of a 2-h splicing reaction, and is labelled with the name of the construct and the position downstream of the branch point, and the sequence of the first AG-containing trinucleotide

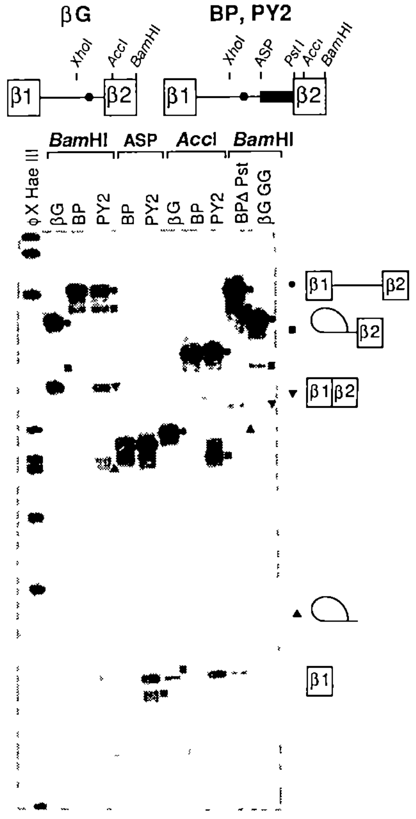

FIG 4 Branch point and 3′ splice site selection in β-globin transcripts Transcripts were produced containing the first intron of the human β-globin gene and the flanking exons (βG) Template BP was derived from the parent βG-containing construct RR108 by inserting nucleotides 111–213 of the *α-TM* intron (shown by the thick line in the schematic diagram) between the β-globin polypyrimidine tract and 3′ exon Template BP was constructed by PCR amplification of a fragment from the *Xho*I site upstream of the β-globin branch point, up to, but not including the 3′ splice site AG, followed by an ASP718 and an *Eco*RV site, nucleotides 111–213 of the *TM* intron with the final 6 nt changed to a *Pst*I site, and globin exon 2 up to the *Acc*I site The *Xho*I-*Acc*I fragment was cloned back into the parent globin framework Construct PY2 was derived from BP by the replacement of the six purines between the β-globin branch site and the ASP718 site with T residues, by oligonucleotide-directed mutagenesis with PCR amplification Construct BPΔPst had the 3′ splice site of BP removed by blunt-ending the *Pst*I site and re-ligating Construct βG GG was obtained by deleting between the *Kpn*I (ASP) and *Pst*I sites of BP leaving a single A-to-G change in the 3′ splice site of βG Transcripts were produced from templates linearized at the *Bam*HI, *Acc*I and ASP718 sites The products of 2-h splicing reactions are shown The identities of all lariat bands were verified by debranching and the branch point used by βG and BP (shown by the dot) was verified by primer extension with reverse transcriptase

Crystal structure of chaperone protein PapD reveals an immunoglobulin fold

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The chaperone protein PapD mediates assembly of pili in *Escherichia coli* Its polypeptide chain folds into two immunoglobulin-type domains that are homologous in sequence to the human lymphocyte differentiation antigen Leu-1/CD5

UROPATHOGENIC *Escherichia coli* carries an operon of 11 genes[1-3] for synthesis and assembly of pap pili which mediate attachment of the bacteria to eucaryotic cell surfaces One of these genes, *PapD*, codes for a chaperone protein which mediates assembly of pili by forming soluble multimeric complexes with pili subunits as an intermediate step in the assembly process[4] We report here the crystal structure of PapD to 2 5 Å resolution

The crystal structure of PapD

The PapD protein was crystallized in an orthorhombic space group $P2_12_12_1$ with cell dimensions $a = 58$ 2 Å, $b = 64$ 0 Å and $c = 67$ 0 Å, with one molecule in the asymmetric unit[5] The processed protein was purified from an *E coli* expression system and included the region of the gene encoding the signal peptide X-ray data to 2 5 Å resolution were collected on a Xentronics area detector A multiple isomorphous replacement (MIR)/anomalous electron density map was calculated using three heavy metal derivatives giving an average figure of merit of 0 66 Phasing statistics are given in Table 1 The polypeptide chain was traced using the Bones option in Frodo[6] The amino-acid sequence[4], as predicted from the complementary DNA sequence, was fitted into the map using the aromatic side chains as reference points (Fig 1) The structure has been initially refined using Xplor[7] on an Alliant Fx/4 computer giving an *R*-factor of 0 24 with no water molecules added The current model of 218 residues leaves no regions of the MIR map unassigned The amino acids of the model are numbered from 22 to 239 to conform to the numbering of the published sequence with the leader peptide (1-21) included Coordinates of the current model have been deposited in the Brookhaven Protein Data Bank

The structure of PapD is built up from two globular domains oriented such that the complete molecule has the shape of a boomerang (Figs 2 and 4) The two domains have very similar structures with an antiparallel beta-barrel structure formed by two beta sheets packing against each other The topology of each domain is the same as the constant domain of immunoglobulin molecules, comprising seven anti-parallel beta strands, A to G (ref 8) Both domains lack the disulphide bridge between beta strands B and F which is usually but not always found in immunoglobulin domains[8] The residues that form the beta strands are given in Fig 2*b*

The N-terminal domain comprises residues 22-121 Two long loop regions are present at one end of the barrel between strands C and D (residues 62 to 73) and between strands E and F (residues 94-106) The other loop regions are either reverse turns or short loops comprising three to five residues

Residues 153 to 239 form the C-terminal domain In addition to the seven beta strands A to G of the immunoglobulin fold there is a short beta strand H at the C-terminal end of the polypeptide chain which is positioned between, and is antiparallel to, beta strands G and A (Fig 2) This beta strand is firmly linked to strand G by a disulphide bridge between Cys 228 and Cys 233 The four residues between these cysteines

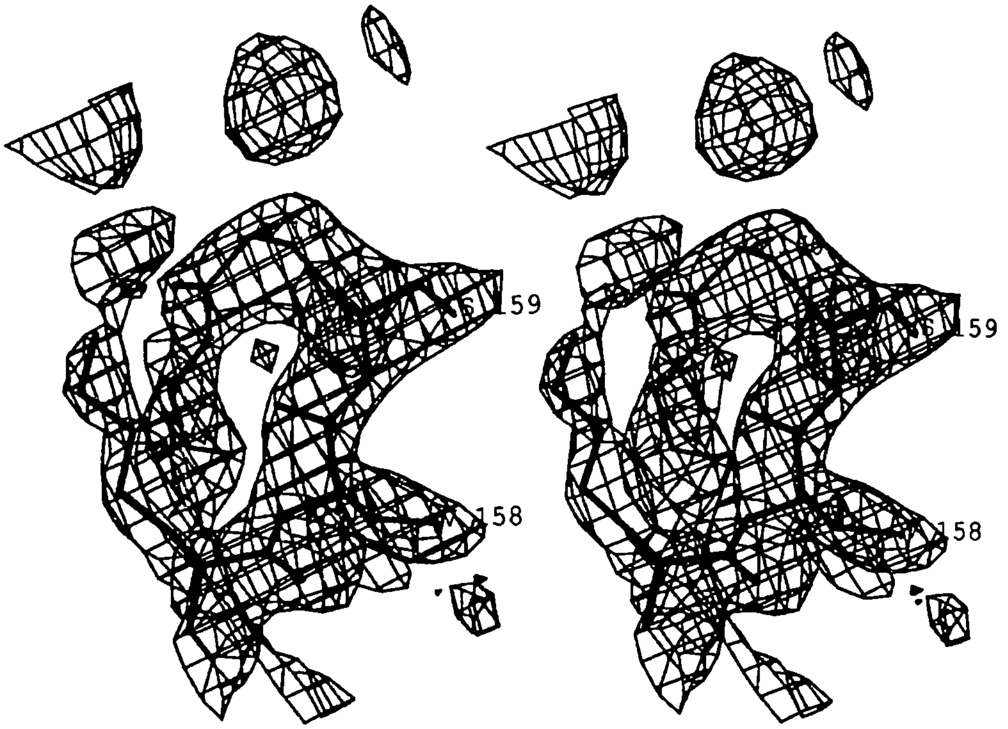

FIG 1 Representative electron density map showing a reverse beta-turn between strands A and B in the C-terminal domain

TABLE 1 Crystallographic data

Space group P $2_12_12_1$ (*a*=58 2 Å, *b*=64 0 Å, *c*=67 0 Å)

Data set	Native	K_2PtCl_4	$K_2Pt(SCN)_4$	$Pt(NH_3)_2Cl_2$
No of observations	20,820	20,429	20,375	15,100
No of unique reflexions	6,353	6,456	7,630	6,633
Possible reflexions (%)	69 2	69 2	81 4	68 4
R merge (%)	5 3	5 5	6 6	7 4
Mean fractional isomorphous change (%)	—	20 4	15 5	22 3
No of heavy atom sites		5	4	5
F_h/E r m s		3 20	1 26	2 19
R cullis		0 46	0 78	0 51

Resolution (Å)	17 0	9 3	6 4	4 9	3 9	3 3	2 8	2 5	total
No of reflections	35	169	387	682	1,011	1,363	1,587	819	6,053
Figure of merit	0 81	0 89	0 89	0 82	0 73	0 66	0 56	0 45	0 66

R merge$=\sum_h (I_s-\bar{I})/\sum_h \bar{I}$

F_h/E r m s F_h=heavy atom structure factor, *E* is the residual lack of closure

R cullis$=\sum |F_{ph}(obs)-F_{ph}(calc)|/\sum |F_{ph}(obs)-F_p(obs)|$

PapD was isolated from the periplasm of an *E coli* strain containing a cloned *papD* gene under the influence of the *tac* promoter[4], thereby forcing the bacteria to overproduce the protein PapD was collected from the periplasmic space and purified by the method of Lindberg *et al*[4] with some modifications[5] Crystals of PapD were obtained by the hanging drop method of vapour diffusion using a protein concentration of 12 mg ml^{-1} (ref 5), and 15% polyethylene gylcol 8000 as precipitating agent Crystals appeared after a few days and usually grew to a size of 0 5 mm in all directions X-ray data were initially collected to 5 0 Å on a single counter four circle diffractometer Analysis of difference Patterson maps was undertaken using the program RSPS (written by Stefan Knight), which provided the heavy atom positions for the major site in the three derivatives used and indicated some minor sites of low occupancy The heavy-atom positions were further refined using the program Heavy in the CCP4 program package from Daresbury UK Difference Fourier maps, calculated by the program Protein[21], revealed some additional minor sites X-ray data to 2 5 Å resolution were collected for those derivatives which gave good refinement statistics on a Xentronics multiwire area-detector[22], using the software system described by Blum *et al*[23]

form a short hairpin loop between strands G and H The sulphur atoms of this disulphide bridge (Fig 3) are oriented towards the hydrophobic core of the barrel and do not react with heavy metal reagents The linker region between the domains, residues 122 to 152 contains a number of hydrophobic side chains which form a hydrophobic core in the central region of the molecule Additional residues in this hydrophobic core are provided by loop regions from both domains Secondary structure elements in relation to the amino-acid sequence of PapD are given in Fig 5

Structural similarity with immunoglobulins

To determine whether the topological similarity between the PapD and immunoglobulin domains also reflects structural similarity, we have compared these domain structures using the HOMO program[9] The alpha carbon atoms of the two PapD domains were superimposed with each other and with the constant domain CH2 of an IgG Fc-fragment[10] We found that 50 alpha carbon atoms of the PapD domains superimpose with each other with a root mean square error (r m s) of 3 5 Å, showing that only about half of the residues that build up the two domains are structurally equivalent These residues build up the core of the domains An r m s value of 3 5 Å for superposition of the cores of two structurally equivalent domains is compatible with the fact[4] that there is no amino-acid homology between these two domains[11]

In contrast to the above results, the cores of 50 alpha carbon atoms of PapD domains 1 and 2 superimpose with CH2 with r m s values of 2 1 and 2 5 Å respectively Each PapD domain is thus more similar in structure to the immunglobulin domains than they are to each other They seem to be two different variations of the CH2 core structure In both these PapD domains the beta strands are arranged in two distinct beta sheets, similar to the arrangement in all immunoglobulin structures[8] and different from that in other structures of the same topology such as superoxide dismutase, which is more like a barrel[12]

Sequence homology with CD5-antigen

There is no significant amino-acid sequence similarity between CH2 and the PapD domains To examine if other members of the immunoglobulin superfamily are homologous to PapD we searched in the NBRF protein-sequence data base (Protein Identification Resource), and found that amino-acid residues −4 to 252 of the Leu-1 human lymphocyte differentiation antigen also called CD5 (ref 13), could be aligned with the sequence of the complete PapD molecule giving a 26% identity All gaps

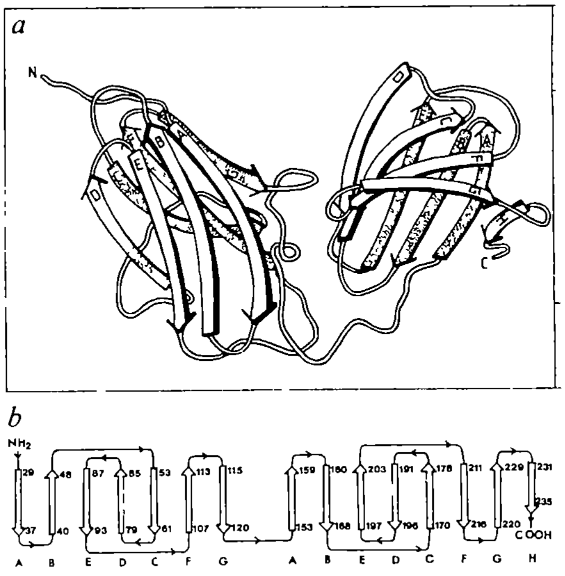

FIG 2 *a*, Schematic diagram of the PapD molecule, showing the arrangement of the beta strands in the two domains Strands A B E form one beta sheet and strands C F G form a second beta sheet which is packed against the first sheet Dotted strands belong to the sheet at the back of the figure Beta strand D of the N-terminal domain belongs to both beta sheets by forming hydrogen bonds to strand C at the beginning and to strand E at its end *b*, Topology diagram of the course of the polypeptide chain of PapD Residue numbers that form approximate beginnings and ends of the beta strands are given starting with residue 22 which is the first residue after the signal peptide and another area of three acidic residues on the C-terminal domain (E185, E186, E188). We suggest that this region is involved in binding the pili subunits. This model of the interaction area forms the basis for specific mutagenesis work, now in progress.

The amino-acid-sequence homology between *E. coli* PapD and human Leu-1/CD5 raises some puzzling evolutionary questions. The rate of evolution in the CD5 proteins is quite fast, as the functionally homologous CD5 proteins in human (Leu-1)

show rather low sequence identity, from 43% in domain 1 to 58% in domain 3. The possibility of divergent evolution from a common ancient bacterial ancestor therefore seems to be excluded. It is more likely that horizontal gene transfer has occurred at a much later stage in evolution. Genes involved in eukaryotic cell–cell interactions may have been recruited by bacteria to aid in their attachment to eukaryotic cells. Such gene transfer would also explain why a member of the immunoglobulin superfamily has been found in *E. coli* bacteria. □

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1. Norgren, M. *et al. EMBO J.* **3,** 1159–1165 (1984).
2. Lindberg, F. P. *et al. Nature* **328,** 84–87 (1987).
3. Hultgren, S. J. *et al. Proc. natn. Acad. Sci. U.S.A.* **86,** 4357–4361 (1989).
4. Lindberg, F. P. thesis, Umeå Univ. (1987).
5. Holmgren, A., Brånden, C.-I., Lindberg, F. & Tennent, J. M. *J. molec. Biol.* **203,** 279–280 (1988).
6. Jones, T. A. & Thirup, S. *EMBO J.* **5,** 819–822 (1986).
7. Brunger, A. T., Kurlyan, J. & Karplus, M. *Science* **235,** 458–460 (1987).
8. Williams, A. F. & Barclay, A. N. *A. Rev. Immun.* **6,** 381–405 (1988).
9. Rao, S. T. & Rossmann, M. G. *J. molec. Biol.* **76,** 241–256 (1973).
10. Deisenhofer, J. *Biochemistry* **20,** 2361–2370 (1981).
11. Chothia, C. & Lesk, A. M. *EMBO J.* **5,** 823 (1986).
12. Richardson, J. S. *et al. J. molec. Biol.* **102,** 221–235 (1976).
13. Jones, N. H. *et al. Nature* **323,** 346–349 (1986).
14. Huang, H.-J. S. *et al. Proc. natn. Acad. Sci. U.S.A.* **84,** 204–208 (1987).
15. Williams, A. F. *Immun. Today* **8,** 298–303 (1987).
16. Hemmingsen, S. *et al. Nature* **333,** 330–334 (1988).
17. McMullin, T. W. & Hallberg, R. L. *Molec. cell. Biol.* **8,** 371–380 (1988).
18. Goloubinoff, P. *et al. Nature* **337,** 44–47 (1989).
19. Ellis, R. J. *Nature* **328,** 378–379 (1987).
20. Ostermann, J. *et al. Nature* **341,** 125–130 (1989).
21. Steigemann, W. thesis, Technische Univ. Munich (1974).
22. Durbin, R. M. *et al. Science* **232,** 1127–1132 (1986).
23. Blum, M. *et al. J. appl. Crystallogr.* **20,** 235–242 (1987).

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LETTERS TO NATURE

The distribution of galaxies in the direction of the 'Great Attractor'

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THE discovery of galaxy streaming motions of up to 600 km s^{-1} towards Hydra–Centaurus has led to the suggestion[1] that this motion is induced by a concentration of galaxies (the 'Great Attractor') centred on galactic coordinates $l = 307°$, $b = 9°$ at a distance corresponding to $4{,}350 \pm 350$ km s^{-1} in the Hubble Flow. Here we present a map of ~17,000 galaxies down to $B_J = 17^m$ in an extended region of the sky in this direction. The clusters of galaxies identified from the map are shown to constitute two distinct concentrations in redshift (see, for example, ref. 2). We explore the possibility that the further of the two concentrations, centred near $l = 312°$, $b = 31°$, at a mean redshift of 14,000 km s^{-1}, is a major contributor to the peculiar motion of the Local Group. This is unlikely because the contribution to the dipole anisotropy of extragalactic light due to galaxies in this region, beyond a diameter limit of 1.3 arcmin, is about 10% of that due to nearer galaxies, and the concentration would need to have an extremely high mass-to-light ratio. Superclusters as massive as this would cause appreciable anisotropies in the cosmic microwave background radiation.

We have completed a survey of galaxies in an extended region around the proposed 'Great Attractor', using glass copies of UK Schmidt Telescope (UKST) Sky Survey plates (emulsion IIIa-J), which have been scanned by the SERC Automatic Photographic Measuring (APM) facility in Cambridge. The area covered (Fig. 1) consists of 103 such plates, each covering 5° × 5° of the sky. After preliminary star-galaxy separation, all galaxy candidates have been visually inspected, and morphological types assigned to the galaxies found. The magnitudes of galaxies on different plates have been reduced to a consistent system by using the galaxies in the $\gtrsim 1°$ overlaps between neighbouring plates. A catalogue of galaxies (the Hydra-Centaurus catalogue), which is $\gtrsim 97\%$ complete down to a magnitude limit of $B_J \simeq 17^m$, has been compiled. It consists of 16,963 galaxies, for which positions, relative magnitudes, diameters and various profile parameters have been measured: >80% of these being catalogued for the first time.

The galaxies in the Hydra-Centaurus catalogue are plotted in an equal-area projection in Fig. 1*a*. All the Abell-Corwin-Olowin (ACO) clusters[3] found in this region, along with some poor clusters, are indicated in Fig. 1*b*, and listed in Table 1. These figures can be compared with Fig. 2, which is a plot of all galaxies >1 arcmin in this region of the extragalactic sky, from existing catalogues (ESO, MCG and UGC: see Fig. 8 of ref. 1).

As large-scale redshift surveys in this area[4,5] have concentrated mostly on galaxies in the ESO catalogue (ref. 15) not many redshifts are found in the literature for galaxies beyond ~6,000 km s^{-1}. However, many clusters in this region out to 20,000 km s^{-1} have been studied in detail[6-10]. Figure 3 shows that almost all of these clusters belong to two distinct groups, with none between 5,000 km s^{-1} and 10,000 km s^{-1} in the area of interest. The nearer group is what is commonly known[11] as the 'Hydra-Centaurus Supercluster'. On the other hand, all the 14 clusters lying between $10{,}000 < v < 16{,}500$ km s^{-1} lie within 10° of A 3558 (Shapley 8: $l = 312°$, $b = 31°$), the mean redshift being $\langle v \rangle = 13{,}620$ km s^{-1}. The existence of an excessive concentration of galaxies in this region was first discovered by Shapley[12], and is referred to here as the 'Shapley Concentration'. This concentration has recently been pointed out in the distribution of galaxies[6], ACO rich clusters[2] and X-ray clusters[13].

Because both gravity and luminosity fall as the inverse square of the distance, the dipole moment of the distribution of galaxies (weighted by a function of their luminosities) has often been used[11,14] to estimate the peculiar acceleration of the Local Group. Using the expression $P = \sum_i D_i^2 \hat{r}_i$ as such an estimator, where D_i is the major angular diameter of a galaxy i, normalized to the ESO system[15] and corrected for galactic extinction[16], the 'optical dipole' has been estimated to be $P_{>1.3'} \approx 17{,}300$ (ESO arcmin)^2, directed towards $l = 257°$, $b = 34°$, from an all-sky catalogue of 16,886 galaxies having $D_{\mathrm{ESO}} > 1.3'$ (ref. 14, Table 1).

Here, we attempt to estimate the contribution of the galaxies in the Hydra-Centaurus catalogue, beyond the above diameter limit, to the optical dipole. To do this, we have calculated the value of

$$\mathscr{P}_{<1.3'} = \sum_{\text{fields}} \left[\left(\sum_j D_j^2 \hat{r}_j \right) - \langle \Delta^2_{\mathrm{SGP}} \rangle \hat{r}_{\mathrm{f}} \right],$$

where $\hat{r}_f$ refers to the unit vector in the direction of the centre of a UKST field and $\hat{r}_j$ the unit vector towards all galaxies j, with major (angular) diameter D_j in units of ESO arcmin, in that field. The dipole is normalized to the value of $\langle\Delta^2_{\rm SGP}\rangle$, which is the mean $\sum D^2$ per field derived from the 15,151 galaxies down to the same magnitude limit, compiled by the APM survey[17] from 185 UKST fields around the South Galactic Pole (Loveday, unpublished thesis, Univ. of Cambridge, 1989). In both cases, all galaxies in the range $0.5' \leq D_{\rm ESO} \leq 1.3'$ have been used. The underlying assumptions here are (1) that the dipole summed over the rest of sky is negligible, and (2) that the SGP value is a fair representation of the rest of the sky at that magnitude limit, being averaged over a very large area of sky (~1 sr) far away from the Hydra–Centaurus region. As the ESO catalogue and the APM catalogues (SGP, HC) measure their diameters to different isophotes, a mean linear relation between their diameters is estimated using the galaxies in common between the ESO catalogue and the APM catalogues. The angular diameters have been corrected for galactic extinction (ref. 16) A_B by a factor of $10^{0.2A_B}$.

For the 12,325 galaxies in the Hydra–Centaurus catalogue (103 UKST fields) satisfying $0.5' \leq D_{\rm ESO} \leq 1.3'$, we derive $\mathscr{P}_{<1.3'} \approx 3{,}242$ (ESO arcmin)^2, directed towards $l = 310°$, $b = 39°$, which is 19% of $\mathscr{P}_{>1.3'}$. One can, however, argue that a significant fraction of galaxies smaller than 1.3′ are actually intrinsically smaller galaxies associated with the known concentration of closer ESO galaxies. To estimate the effect of sample incompleteness that is due to a cutoff in apparent diameter, we assume a 'Light Incompleteness Function' of the form[11]

$$\mathscr{L}(d) = \frac{1 + t/2}{(1 + t/5)^4},$$

where $\mathscr{L}(d)$ gives the fraction of total luminosity emitted by galaxies of 'diameter' $\geq d$; and $t = (d/6{,}538)^2$, where $d = D_{\rm lim} \times \langle v \rangle$ km s^{-1} arcmin, and $D_{\rm lim}$ is the angular diameter (in the ESO system) down to which the sample is complete. To correct for galaxies at the faint end of the luminosity function beyond the diameter limit of a sample, one needs to multiply $\mathscr{P}$ by $\mathscr{L}^{-1}$.

It has been shown that more than half of the dipole that is due to galaxies larger than 1′ (ref. 11) comes from galaxies with $v < 2{,}000$ km s^{-1}, and 80% from those with $v < 4{,}000$ km s^{-1}. For the sake of estimating $\mathscr{L}^{-1}$, we assume that half of $\mathscr{P}_{>1.3'}$ comes from $\langle v \rangle = 2{,}000$ km s^{-1} and the other half from $\langle v \rangle = 3{,}500$ km s^{-1}, which gives $\mathscr{L}^{-1} = 1.11$. This indicates that more than half of $\mathscr{P}_{<1.3'}$ as estimated above comes from smaller galaxies that are in the nearer concentration. Accordingly, a similar correction should be applied to the further concentration

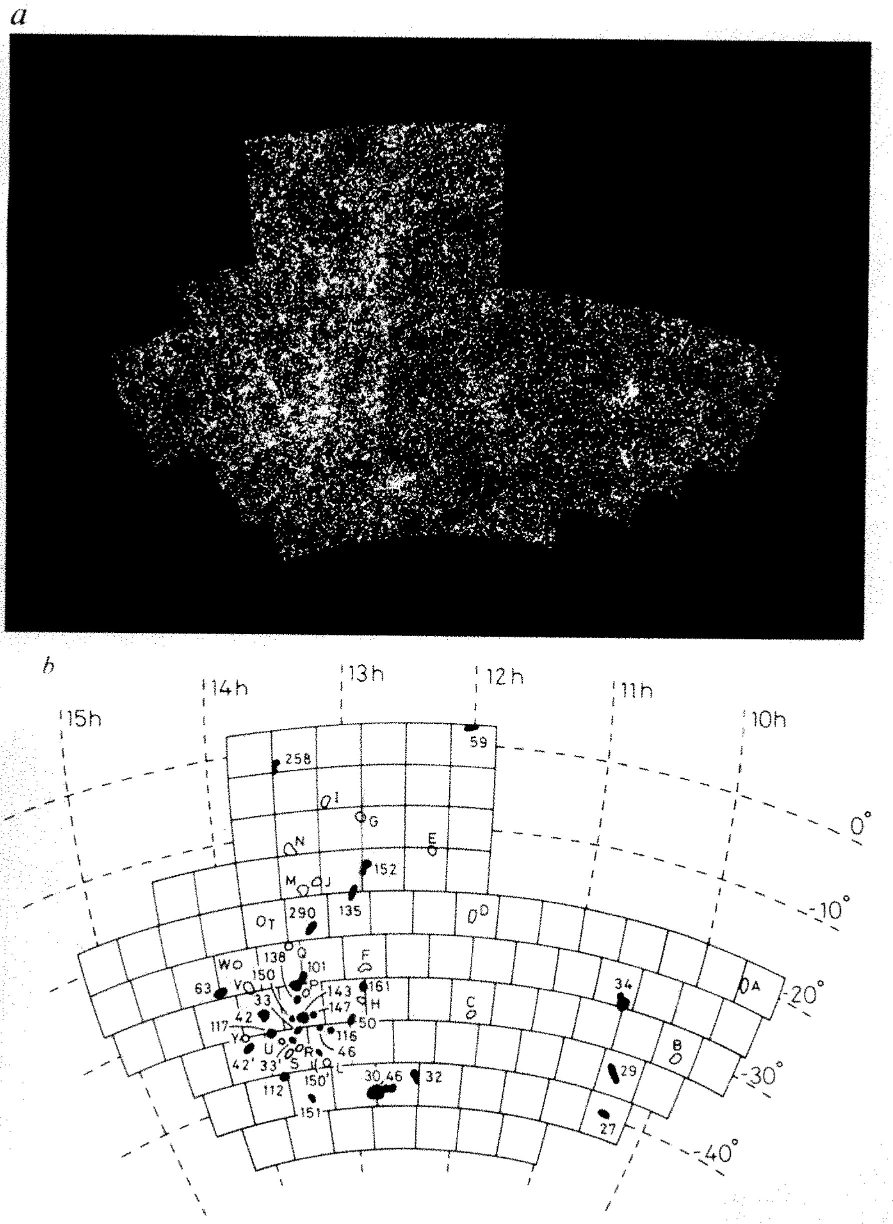

FIG. 1 *a*, An equal-area plot of the 16,963 galaxies in the Hydra–Centaurus catalogue down to $B_j = 17^m$. Each point represents a galaxy. Large chains of rich clusters, threaded with filamentary structures are clearly seen, especially in the region 12 h 40 min < α < 13 h 50 min, −35° < δ < −25°. *b*, The boundaries of the 103 UKST fields surveyed are shown, together with the positions of the clusters of galaxies identified in *a* and listed in Table 1. The numbers refer to the mean redshift of the clusters, where available, in units of 100 km s^{-1}. A right ascension–declination grid shows the position in the sky.

as well, to account for galaxies missed by the diameter limit of the Hydra–Centaurus catalogue. Because the latter is magnitude limited, its diameter limit is not well defined; however, we believe that it is fairly complete down to $D_{\rm ESO} = 0.5'$. Assuming that all the excess galaxies in the diameter range $0.5' \leq D_{\rm ESO} \leq 1.3'$ that belong to the further concentration are at $\langle v \rangle = 13{,}620\ {\rm km\ s^{-1}}$, we get $\mathscr{L}^{-1} = 1.42$. This means that the galaxies that are beyond a redshift of about $5{,}000\ {\rm km\ s^{-1}}$ contribute only $1.42 \times (0.19 - 0.11)/1.11 = 10\%$ of the dipole acceleration that is due to those that are nearer.

The fact that the Shapley Concentration lies very close to the proposed position of the 'Great Attractor' has prompted some authors[2,6] to consider the possibility of its being a major contributor to the peculiar motion of the Local Group. Here, we consider the extreme hypothesis that the Shapley Concentration is responsible for the entire peculiar motion of the Local Group (of $570\ {\rm km\ s^{-1}}$), for which it would have to have a mass of $M_{\rm GA} \approx 2.6 \pm 0.8 \times 10^{17}\ h^{-1}\ M_{\odot}$ at a distance of $136\ h^{-1}$ Mpc ($H_0 = 100\ h\ {\rm km\ s^{-1}}$). If all the 3,390 galaxies belonging to the Hydra–Centaurus catalogue, within a radius of 10° of Shapley 8 ($24\ h^{-1}$ Mpc at a distance of $136\ h^{-1}$) and smaller than $D_{\rm ESO} = 1.5'$, were placed at a distance of $136\ h^{-1}$ Mpc, and their magnitudes corrected for galactic extinction[16], then the total luminosity would be $L_{\rm J} = 4.1 \times 10^{13}\ h^{-2}\ L_{{\rm B},\odot}$, leading to $M/L_{\rm B} \approx M/L_{\rm J} \approx$

TABLE 1 Clusters of galaxies found in the Hydra–Centaurus catalogue

	No.	Position (1950)‡		$\langle v_r \rangle$§	σ‖	N_v¶	Other names**
*	ACO†	RA	Dec	km s^{-1}	km s^{-1}		
A	842	9 h 35.9 min	−20° 42′				
B		9 57.3	−31 9				
27		10 23.0	−39 31	2,725	139	8	N 3244
29		10 28.4	−34 59	2,860	546	20	Antlia, P 636
34	1060	10 34.5	−27 16	3,417	623	103	Hydra I
C	3494	11 54.6	−31 53				
D		11 57.6	−20 16				
59		12 1.9	2 11	5,850	708	13	MKW-4
E	1520	12 17.0	−13 0				
32		12 26.8	−39 10	3,194	268	6	N 4373
30	3526a	12 46.1	−41 2	3,041	586	123	Centaurus 30
46	3526b			4,570	262	57	Centaurus 45
152*	1631	12 50.2	−15 10	13,958	711	71	
F	1633	12 51.5	−26 7				
161	3528	12 51.6	−28 45	16,050	740	39	K 21
G	1635	12 51.7	−8 40				
135*	1644	12 54.6	−17 6	14,202	991	92	
H	3532	12 54.6	−30 6				K 22
50	3537	12 58.3	−32 10	5,010			K 23
I		13 7.7	−7 9				
116	3545	13 8.6	−33 49	11,550			
J		13 12.0	−16 13				
L		13 15.9	−37 0				
290	1709	13 16.0	−21 12	28,980			
46	3554	13 16.7	−33 13	4,590			
150′		13 18.7	−35 32	14,970			K 24, P 729
M	1725	13 21.2	−16 33				
147	3556	13 21.3	−31 24	14,700	590	12	
P	3557	13 22.1	−28 37				
N		13 24.1	−12 0				
101*	1736a	13 24.1	−26 52	10,412	381	34	IC 4255
	1736b			13,815	984	63	
151		13 25.0	−40 52	15,090			P 734
143	3558	13 25.1	−31 14	14,300	1,250	91	Shapley 8
138	3559	13 27.1	−29 16	13,750	600	9	
258	1750	13 28.3	−1 36	25,800		1	
33	3560	13 29.0	−32 58	3,270			
150	3562	13 30.7	−31 25	14,970			
Q	1757	13 30.8	−23 1				K 26?
R	3564	13 31.5	−34 58				
33′	3565	13 33.8	−33 43	3,270			IC 4296
S	3566	13 36.1	−35 18				
T		13 41.3	−19 33				P 741
U		13 41.7	−34 43				P 742
112	3570	13 43.9	−37 40	11,160			
117	3571	13 44.6	−32 37	11,730	1,060	69	
42	3574	13 46.3	−30 3	4,230			K 27
V	3577	13 51.5	−27 36				
W	3578	13 59.7	−24 29				
42′		14 0.7	−33 44	4,200			P 753
Y		14 1.5	−32 43				
63	3581	14 4.6	−26 47	6,270			PKS 1406-267

This table lists the galaxy clusters found in the APM Hydra–Centaurus Survey, indicated in Fig. 1b. The columns indicate: * the marker used in Fig. 1*b* (letters for clusters with no available redshift, numbers refer to redshift of the cluster in 100 km s^{-1}, except for those marked with an asterisk for which the redshifts were updated later); † 'Abell' numbers (from the ACO rich cluster catalogue[3]); ‡ position (1950), §‖ mean redshift with respect to the Local Group, and velocity dispersion, in km s^{-1}, where available (refs 6–10, and Quintana and da Souza, preprint); ¶ the number of galaxies used in estimating $\langle V_r \rangle$ and σ, if known; and ** Alternative names found in the literature (P, ACO poor clusters[3], K, Klemola[21]).

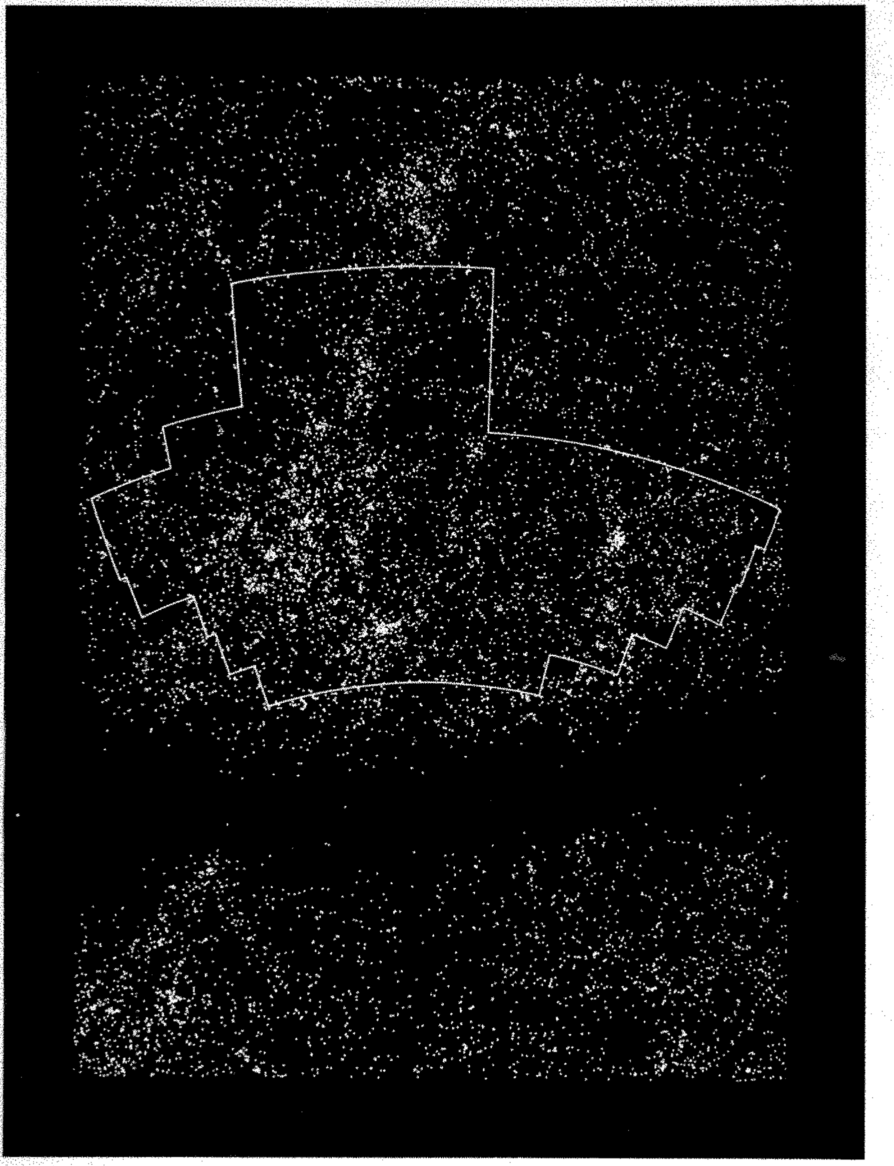

FIG. 2 An equal-area plot of all galaxies in the survey region and its immediate neighbourhood, as found in existing catalogues (a merged version of the ESO, MCG, UGC catalogues as used in Fig. 8 of ref. 1). The area enclosed by the survey region (outline indicated) contains 1,220 galaxies larger than $D_{\mathrm{ESO}} > 1.3'$. The cluster to the north of the survey area is the Virgo Cluster. The Supergalactic Plane runs across the plot, on either side of the galactic plane, the latter showing up as an empty 'zone of avoidance'. The nearby clusters ($\langle v_r \rangle <$ 5,000 km s^{-1}, such as Hydra I and Centaurus) are easily seen, but not those belonging to the further Shapley Concentration.

6,300 h (for comparison: $M/L_B \approx 1{,}500\,h$ for an $\Omega_0 = 1$ Universe). This makes the Shapley Concentration unlikely to be the Great Attractor, as it would also imply (1) that mass does not follow light, and dark matter prefers to reside in clusters and superclusters of galaxies rather than in the field, and furthermore, (2) that superclusters are capable of having higher mass-to-light ratios ($M/L_B \approx 6{,}000\,h$) than the richest clusters of galaxies (for example, Perseus: $M/L_B \approx 850\,h$). Both of these are against biasing arguments.

Furthermore, if the Shapley Concentration were a major source of the dipole acceleration of the Local Group, one would expect peculiar velocities to continue to rise beyond the nearer concentration of galaxies. However, preliminary results[18] from Dressler and Faber's survey in this region indicate that peculiar velocities of galaxies decrease beyond 4,000 km s^{-1}. The Hydra–Centaurus catalogue presented here can serve as a source for more extensive surveys of this kind.

Massive 'attractors' can have an appreciable effect on the cosmic microwave background radiation (CMBR). Rees and Sciama[19] showed that a nearby expanding (contracting) density enhancement, such as a supercluster, will cause CMBR photons passing through it to be blueshifted (redshifted) relative to photons reaching us from other directions, causing an anisotropy in the CMBR on the scale of the size of the supercluster (a few degrees). If the entire mass of $2.7 \times 10^{16}\,h^{-1}\,M_\odot$ of the Great

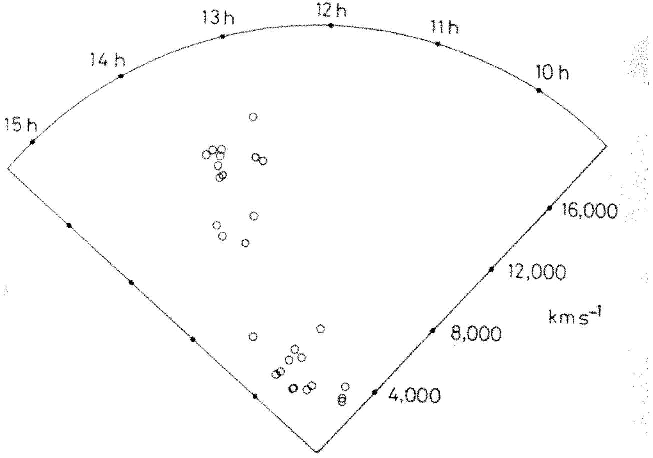

FIG. 3 A wedge diagram of galaxy clusters with known redshifts, showing the position on the sky (right ascension) against mean redshift. The plot clearly shows the existence of two distinct groups of clusters. The nearer group is known as the 'Hydra–Centaurus Supercluster', consisting of three subgroups: (1) the 'Hydra' subgroup at a distance of $\langle v \rangle = 3{,}000$ km s^{-1}, (2) the 'Centaurus' subgroup at $\langle v \rangle = 3{,}200$ km s^{-1}; and (3) the Klemola 27 subgroup at $\langle v \rangle = 4{,}500$ km s^{-1}. The further group, between $10{,}000 < v <$ 16,500 km s^{-1}, is the 'Shapley Concentration', the mean redshift being $\langle v \rangle = 13{,}620$ km s^{-1}. Note the absence of clusters between 5,000 and 10,000 km s^{-1}, and beyond 3,500 km s^{-1} in the range 9 h < RA < 12 h 30 min.

Attractor[1], at a distance of 4,350 km s^{-1}, were confined to a uniform sphere of radius 15 h^{-1} Mpc ($\delta\rho/\rho_0 = 6\Omega_0^{-1}$), it would produce $|\Delta T/T| \approx 2\times10^{-6}$ for $\Omega_0 = 1$. On the other hand, if the Shapley Concentration at 13,600 km s^{-1} were the Great Attractor, and could be represented as a spherical supercluster of mass $2.6\times10^{17}\,h^{-1}\,M_\odot$ within a radius of 20 h^{-1} Mpc ($\delta\rho/\rho_0 = 27\Omega_0^{-1}$), it would lead to a Rees–Sciama effect of the order of $|\Delta T/T| \approx 4\times10^{-5}$. This is close to the observable limits of the Davies *et al.* beam-switching experiment[20], which in its present configuration cannot point so far south in declination.

Both the values of the luminosity and the optical (diameter) dipole of the Shapley Concentration indicate that it could be responsible for $\lesssim$10% of the net acceleration of the Local Group, the rest being due to much nearer galaxies. It would also have a slightly greater dynamical effect on the nearer galaxy concentration, which in turn retains its status as the principal 'Great Attractor' as far as the Local Group is concerned. ☐

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1. Lynden-Bell, D. *et al. Astrophys. J.* **326,** 19–49 (1988).
2. Scaramella, R., Baiesi-Pillastrini, G., Chincarini, G., Vettolani, G. & Zamorani, G. *Nature* **338,** 562–564 (1989).
3. Abell, G. O., Corwin, H. G. & Olowin, R. P. *Astrophys. J. Suppl.* **70,** 1–138 (1989).
4. Da Costa, L. N. *et al. Astr. J.* **91,** 6–12 (1986).
5. Dressler, A. *Astrophys. J.* **329,** 519–526 (1988).
6. Melnick, J. & Moles, M. *Rev. Mexicana Astr. Astrof.* **14,** 72–76 (1987).
7. Hopp, U. & Materne, J. *Astr. Astrophys. Suppl.* **61,** 93–106 (1985).
8. Lucey, J.R., Currie, M. J. & Dickens, R. J. *Mon. Not. R. astr. Soc.*, **221,** 453–472 (1986).
9. Metcalfe, N., Godwin, J. G. & Spenser, S.D. *Mon. Not. R. astr. Soc.* **225,** 581–590 (1987).
10. Dressler, A. & Schectman, S. A., *Astr. J.* **95,** 284–297 (1988).
11. Lynden-Bell, D., Lahav, O. & Burstein, D. *Mon. Not. R. astr. Soc.* (in the press).
12. Shapley, H. *Harvard Coll. Obs. Bull.* **874,** 9–12 (1930).
13. Lahav, O., Edge, A. C., Fabian, A. C. & Putney, A. *Mon. Not. R. astr. Soc.* **238,** 881–895 (1989).
14. Lahav, O., Rowan-Robinson, M. & Lynden-Bell, D. *Mon. Not. R. astr. Soc.* **234,** 677–701 (1988).
15. Lauberts, A. *The ESO/Uppsala Survey of the ESO-B Atlas* (European Southern Observatory, Garching, 1982).
16. Burstein, D. & Heiles, C. *Astr. J.* **87,** 1165–1189 (1982).
17. Maddox, S. J., Efstathiou, G. & Loveday, J. in *Large Scale Structures of the Universe*, Proc. IAU 130 (eds Audouze, J., Pelletan, M.-C. & Szalay, A.) 151–160 (Kluwer, Dordrecht 1987).
18. Dressler, A. in *Proc. 14th Texas Symp. Relativistic Astrophys.* (New York Acad. Sci., in the press).
19. Rees, M. J. & Sciama, D. W. *Nature* **217,** 511–516 (1968).
20. Davies, R. D. *et al. Nature* **326,** 462–465 (1987).
21. Klemola, A. R. *Astr. J.* **74,** 804–806 (1969).

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Pre-biotic organic matter from comets and asteroids

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SEVERAL authors[1–3] have suggested that comets or carbonaceous asteroids contributed large amounts of organic matter to the primitive Earth, and thus possibly played a vital role in the origin of life. But organic matter cannot survive the extremely high temperatures ($>10^4$ K) reached on impact, which atomize the projectile and break all chemical bonds. Only fragments small enough to be gently decelerated by the atmosphere—principally meteors of 10^{-12}–10^{-6} g—can deliver their organic matter intact[4]. The amount of such 'soft-landed' organic carbon can be estimated from data for the infall rate of meteoritic matter. At present rates, only ~0.006 g cm^{-2} intact organic carbon would accumulate in 10^8 yr, but at the higher rates of $\sim4\times10^9$ yr ago, about 20 g cm^{-2} may have accumulated in the few hundred million years between the last cataclysmic impact and the beginning of life. It may have included some biologically important compounds that did not form by abiotic synthesis on Earth.

Figure 1 shows the present estimates of the meteoritic mass influx on Earth[5], including meteors, meteorites and crater-forming bodies (asteroids and comets). The meteor data[6] cover only the past few decades, but as they agree[7] with noble-metal measurements in deep-sea sediments[5,8] and lunar soils[9], they should be representative of the past $\sim3.6\times10^9$ yr. The cratering curve should be similarly representative, being based in part on crater counts on lunar maria that are 3.3–3.8×10^9 yr old (ref. 10).

Now consider the size ranges in which organic matter can be preserved (shaded areas in Fig. 1). At very small masses, ultraviolet photolysis alters and destroys the organic matter. For the sake of definiteness I set the limit for such destruction at $<10^{-12}$ g. At higher masses, atmospheric friction heats the particle to increasingly higher temperatures. The nominal threshold for boiling of silicates is $>10^{-6}$ g, but it depends strongly on density, velocity, entry angle and fragmentation[6,11]. Well preserved interplanetary dust particles (containing noble gases, nuclear tracks and organic matter) range up to 50 μm in size[11] and as many of these may be fragments of larger aggregates or particles, I adopt 10^{-6} g as the upper limit for survival of organic matter. In the next few intervals, much or all of the mass is lost by ablation[6,11], and any remainder is strongly heated by conduction. Ablation calculations and thermoluminescence data show that pyrolytic temperatures (>600 °C) generally extend <1 mm into the meteorite[12], and thus survival of organic matter—at least for low-velocity objects—becomes possible at larger masses, say 10 g. At still larger masses, atmospheric deceleration becomes progressively less effective, causing increasing destruction in the atmosphere[13] or on impact. A reasonable upper limit is 10^8 g, as meteorites of that mass usually reach the ground with a significant fraction of their cosmic velocity. (Also, the largest stony meteorite showers have masses of only a few tonnes.)

It is useful to estimate the accretion rate not only of organic C but also of total C. The first four lines of Table 1 give such data for the main types of meteoritic material. A few numbers require justification.

For meteors, I use the mean carbon value of ~10% for interplanetary dust particles[14] rather than the 24% reported for comet Halley dust[15], as the latter was measured near the comet nucleus where ices had not yet completely vapourized. This value should also apply to dead comets, which are far more likely to strike the Earth than live comets. (The luminous lifetime of an Earth-crossing short-period comet is typically $\sim10^3$–10^4 revolutions, whereas its dynamical lifetime is $\sim10^7$ revolutions.) For the crater-forming bodies, I assume equal numbers of comets, carbonaceous asteroids and non-carbonaceous asteroids. Several lines of evidence indicate a ratio of ~1:1 for the two asteroid classes (see below), but the fraction of comets is poorly constrained, with estimates[10,16] in the range 10–70%. For meteorites, we assume a carbon content of 1.3%, representing 50% ordinary chondrites (median ~0.1% C) and 50% carbonaceous chondrites (range 0.35–4.8% C). Although the latter constitute only 6% of observed stony meteorite falls, this rarity reflects preferential destruction in the atmosphere owing to their friable nature. Carbonaceous objects seem to account for about one-half of the photographic fireballs[17] with final velocities $\leq$8 km s^{-1}, one-half of the asteroids near the 3:1 Kirkwood gap which are thought to be the principal source of meteorites[18], 13 out of 32 Apollo-Amor asteroids[19], and 3 out of 5 chondritic impactors chemically identified at terrestrial craters[20].

Next, I consider the unmelted material. Meteors pass through the atmosphere intact, but meteorites lose much of their mass by ablation. The ablation loss depends strongly on velocity, mass and crushing strength[13]. I shall use 90% for non-carbonaceous chondrites[21] and 99.4% for carbonaceous chondrites (the latter figure is chosen to reduce the proportion of C-chondrites from 50% to 6%).

Obviously, meteors supply most of the organic carbon. It will not be pristine, having been altered by solar ultraviolet and atmospheric heating, but this could make its chemistry more, rather than less, interesting. Meteorites supply only a negligible fraction ($\sim 2\times10^{-5}$) of the total.

The accretion rate of intact organic C, 3.2×10^{8} g yr^{-1}, would give 3.2×10^{16} g, or 0.006 g cm^{-2}, in 10^{8} yr. This amount is either large or small, depending on the frame of reference: although five times greater than the mass of the marine biosphere, it corresponds to a mean carbon concentration in the oceans of only 2×10^{-5} g l^{-1}. But the true value must be higher, because meteorite infall rates were much higher during the first $\sim 10^{9}$ yr of the Earth's history, when the inner planets swept up left-over planetesimals as well as asteroids and comets scattered by the giant planets. To assess the amount of organic carbon contributed by this 'early intense bombardment', we first consider the origin of the Earth's crustal carbon (1.4×10^{23} g; ref. 22).

Less than 10^{-3} of this carbon can come from meteorite infall at present rates over 4.5×10^{9} yr. Even if we count all meteors in Fig. 1 and integrate the comet-asteroid distribution to $m = 8.7\times10^{20}$ g (one impact of this mass or larger is expected in 4.5×10^{9} yr), the total mass is only 2.1×10^{21} g (including 7.4×10^{19} g meteors). For a bombarding population composed equally of comets, carbonaceous asteroids and non-carbonaceous asteroids, the mean C content is 4.2%, which gives a total carbon accretion of 9×10^{19} g in 4.5×10^{9} yr, or only 7×10^{-4} of the Earth's crustal C.

There is good reason to believe that the remaining C (and N, H) was acquired as a 'late veneer' of carbonaceous material during the final stages of the Earth's accretion[23-25]. Not only C, N and H but also other volatile elements (Tl, Pb, B, In, Bi, Cl, Br, I, Ar and Kr) are present in the Earth's crust in approximately C1-chondrite proportions[23,25]. As these elements differ in volatility and atomic weight, it is unlikely that the observed, flat abundance pattern (Fig. 4 of ref. 25) could have arisen from 'impact volatilization'[26,27]—a popular but dubious concept for the final stages of Earth's accretion. It is more likely to reflect an original nebular condensation pattern that was preserved during formation of the crust. (These elements are incompatible with mantle minerals and would strongly concentrate in the crust during mantle differentiation.) The threshold of ~400 K for carbon condensation as complex organic matter[28] is still recognizable in the asteroid belt, where dark, carbonaceous asteroids become dominant at >2.5 AU (ref. 29). About 4.0×10^{24} g of Cl-chondrite-like material ($6.7\times10^{-4}\ M_\oplus$, or a 3.5-km layer) would suffice to account for the 1.4×10^{23} g carbon at the Earth's surface, and the amounts needed for N and H are rather similar (2.9×10^{-4} and $1.5\times10^{-3}\ M_\oplus$).

Two further lines of evidence indicate an infall of this order. First, the uniform enrichment of noble metals (Pd, Re, Os, Ir, Au) in the upper mantle, at $(7.32\pm0.99)\times10^{-3}$ Cl-chondrite abundances[30], apparently implies that highly oxidized, chondritic material fell on the Earth after core formation, leaving its siderophile elements stranded[30,31]. One mantle xenolith showing such enrichment, a sheared garnet lherzolite of particularly primitive composition[32], comes from >150 km depth and so the 'megaregolith' from the late bombardment presumbly extends to at least this depth. This would imply $>5\times10^{-4}\ M_\oplus$ of chondritic material in the upper mantle, which is remarkably consistent with the above values for C, N, H and noble metals (overall mean = $(7.4\pm2.7)\times10^{-4}\ M_\oplus$). Second, the size and age distribution of impact basins on the Moon indicates that the Earth accreted 1.3×10^{-4}-$3.8\times10^{-2}\ M_\oplus$ of material between 3.8 and 4.5 Gyr ago[33]. Some authors have postulated a very large influx of cometary bodies from the region of the outer planets[34], but as only one of the last nine large bodies to strike the Moon[35,36] resembled C-chondrites[30], only part of the bombarding population was carbonaceous.

Although this late accretional component was $\sim 10^{3}$ times greater than the total meteoritic influx at present rates, it probably yielded a smaller proportion of intact organic matter. Like today, the most efficient vehicle for delivery of organic matter would be meteors in the range 10^{-12}-10^{-6} g (or higher if the atmosphere was denser). The meteor fraction probably was at least as large as the present one, that is, ~20% of the total (Table 1), as the disintegration rate of comets should have been similar and the collision rate in the asteroid belt was higher[37]. Much of the soft-landed organic matter would be destroyed or degraded, however, first by the high temperatures of the crust and atmosphere during the most intense stages of the bombardment, then by recurrent vapourization of the ocean and by burial. Material cycled through the >150-km megaregolith would be pyrolysed to elemental C and small molecules such as CO and CH_4, so only organics accumulated in the ocean and in the last few kilometres of the regolith would escape serious degradation.

A reasonable guess—good to perhaps a factor of 3-5—is that only ~1% of the Earth's surface carbon ($\sim 1.4\times10^{21}$ g) accreted under conditions permitting survival of organic carbon. (One per cent of the accretable material ($\sim 5\times10^{22}$ g) would suffice to evaporate the ocean and to deposit several kilometres of ejecta, so accumulation of organic matter probably began only during the last few per cent of the accretion process, but at first with very low efficiency.) If the fraction of meteors, and hence intact organic carbon, was the same as in the most recent infall, that is ~8% (Table 1), this corresponds to 1.1×10^{20} g organic C, or 10^{2} times the total infall in 4.5×10^{9} yr at present rates. This amount is equivalent to 21 g cm^{2} or an oceanic carbon concentration of 0.08 g l^{-1}.

Even 1.1×10^{20} g C is comparable to the 10^{20}-10^{21} g of HCN and HCHO expected from Miller-Urey reactions in the Earth's atmosphere ($H_2/CO_2 = 1$) over 600 Myr (ref. 38). Amounts aside, meteoritic and cometary organic matter may well have

TABLE 1 Accretion rates on Earth

Sources	Mass range (g)	Mass accretion rate (Gg yr^{-1})	Carbon content (%)	Carbon accretion rate (Gg yr^{-1})
Total meteoritic matter	10^{-14}-10^{18}	78	—	4.2
Meteors	10^{-14}-10^{2}	16	10	1.6
Meteorites*	10^{1}-10^{8}	0.058	1.3	7.6×10^{-4}
Crater-forming bodies†	10^{8}-10^{18}	62	4.2	2.6
Unmelted material, contributing organic matter				
Meteors	10^{-12}-10^{-6}	3.2	10	0.32
Meteorites, noncarbonaceous‡	10^{1}-10^{8}	2.9×10^{-3}	0.1	2.9×10^{-6}
Meteorites, carbonaceous§	10^{1}-10^{8}	1.9×10^{-4}	2.5	4.7×10^{-6}

* Assumed pre-atmospheric abundances: 50% carbonaceous chondrites (2.5% C) and 50% noncarbonaceous meteorites (0.1% C).

† Assuming equal mass fractions of dead comets (10% C), carbonaceous asteroids (2.5% C), and noncarbonaceous asteroids (0.1% C).

‡ Assumed ablation loss 90%.

§ Assumed ablation loss 99.4%, to yield observed fraction of 6% carbonaceous chondrites among stony meteorites.

played an important role in pre-biotic evolution, as it formed by different processes (ion-molecule and photochemical reactions in interstellar clouds, grain catalysis in the solar nebula, aqueous chemistry in asteroids) and thus contains a different mix of compounds, including N-heterocyclics, aromatics, amphiphilic compounds, porphyrin-like pigments, and so on, that are not readily made by Miller-Urey reactions[28,39-41].

There are several uncertainties in this estimate: the fraction of carbon-rich objects in the final stages of bombardment, the fraction disintegrated to meteor-sized dust, the onset of safe conditions on Earth, the pressure of the atmosphere, and the net yield of organics from large, friable bodies. A higher pressure would extend aerobraking to higher masses[42], thus raising the soft-landed fraction in Fig. 1. Survival of dust from large, friable bodies would have the same effect. Öpik[43] has calculated that aerodynamic pressure would cause large stony meteorites to disintegrate into a flat sheet of dust and rubble, which would be decelerated to less than crater-forming velocities at masses $\leq 10^{12}$ g. Thomas *et al.*[42] have suggested that part of this debris might be decelerated so gently as to permit survival of organic matter.

At first sight, the empirical data are permissive, or even encouraging. No unmelted material has been found from the Tunguska object, but this may merely reflect its inconspicuous nature and lack of ferromagnetism. Very large amounts of two non-protein amino acids (AIB and ISOVAL) have been reported +0.3 m and −0.5 m from the Cretaceous/Tertiary (K/T) boundary at Stevns Klint, and have been attributed to soft-landed material from a comet[44]. However, these results are in sharp conflict with measurements of Xe in 9 K/T boundary samples from 3 sites, including 6 from Stevns Klint[45,46]. The AIB/Ir ratio is about five times the value in the Murchison C2-chondrite and implies essentially complete survival of the impactor, whereas the Xe/Ir values range from $\leq 6\times10^{-3}$ to 2×10^{-5} of the C-chondrite value, and imply essentially complete destruction. Differences in temperature sensitivity of AIB and Xe cannot explain this discrepancy, as amino acids are far more readily lost than is Xe (seconds at 1,000 K against hours at 1,200-1,500 K), and neither can differences in composition. Comets are ~10-fold enriched in C and N and hence perhaps in amino acids, but they are not likely to be significantly depleted in Ir, as judged from the C-chondrite-like pattern of the micrometeorite component in lunar soils[9] and from the relatively normal abundances of cosmochemically similar elements in comet Halley[15] (Fe = 0.62, Ni = 0.87, Ca = 1.10 of C1-chondrite values). Xenon actually is more abundant in IDPs[47] than in C1 chondrites, by a factor of about ten.

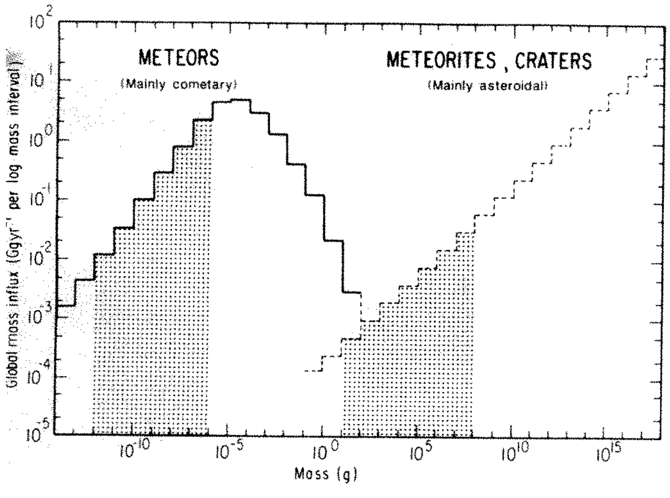

FIG. 1 Infall rate of meteoritic matter on Earth (adapted from ref. 5). Intervals where organic matter can survive passage through atmosphere are shaded. The curve on the right is based on the relation[5] $N=0.54\ r^{-2.1}$ (N = number of impacts per Myr, r = radius in km), for an assumed density of 3 g cm^{-3}. The corresponding mass accretion rate (Gg yr^{-1}) between r_1 and r_2 is $15.83\ (r_2^{0.9}-r_1^{0.9})$.

Furthermore, there are theoretical arguments against survival of unmelted material from comets the size of the K/T impactor (~10^{18} g). During atmospheric passage, such a body would lose only ~10^{-3} of its momentum or mass, unless it had a very small entry angle or very irregular (non-equant) shape[48]. Moreover, the 'ground track' a of the comet's atmospheric trajectory is $h/\tan\alpha$, where h is the altitude where significant fragmentation begins and α is the elevation angle. If $h = 50$ km (ref. 49) and $\alpha \geq 20°$, then a is only ≤ 137 km, much smaller than the ~1,200-km radius of the fireball. Any debris spalled from the comet during atmospheric passage will be engulfed by the fireball and destroyed.

Survival of organic matter against this destruction mechanism becomes possible when the fireball radius is shorter than the ground track, that is at masses $<10^{15}$ g. If a significant fraction of the spalled-off dust is gently decelerated[42] without melting or pyrolysis, then friable bodies of 10^8-10^{12} g may contribute some organic matter. However, at present it seems that the best vehicle for carrying organic matter to Earth is cometary dust formed before atmospheric entry. □

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1. Oró, J. *Nature* **190,** 389-390 (1961).
2. Delsemme, A. H. *Origins Life* **14,** 51-60 (1984).
3. Deamer, D. W., Brack, A., Morowitz, H., Weber, A. & Usher, D. *Origins Life* (in the press).
4. Anders, E., Hayatsu, R. & Studier, M. H. *Science* **182,** 781-790 (1973).
5. Kyte, F. T. & Wasson, J. T. *Science* **232,** 1225-1229 (1986).
6. Hughes, D. W. in *Cosmic Dust* (ed. McDonnell, J. A. M.) 123-185 (Wiley, New York, 1978).
7. Dohnanyi, J. S. *Science* **173,** 558 (1971).
8. Barker, J. L. & Anders, E. *Geochim. cosmochim. Acta* **32,** 627-645 (1968).
9. Anders, E., Ganapathy, R., Krähenbühl, U. & Morgan, J. W. *The Moon* **8,** 3-24 (1973).
10. Shoemaker, E. M. *A. Rev. Earth planet. Sci.* **11,** 461-494 (1983).
11. Brownlee, D. E. *A. Rev. Earth planet. Sci.* **13,** 189-215 (1985).
12. Sears, D. W. *Mod. Geol.* **5,** 155-164 (1975).
13. Baldwin, B. & Shaeffer, Y. *J. geophys. Res.* **76,** 4653-4668 (1971).
14. Schramm, L. S., Brownlee, D. E. & Wheelock, M. M. *Meteoritics* **24,** 99-112 (1989).
15. Jessberger, E. K., Christoforidis, A. & Kissel J. *Nature* **332,** 691-695 (1988).
16. Weissman, P. R. *Geol. Soc. Am. Spec. Pap.* **190,** 15-24 (1982).
17. Wetherill, G. W. & ReVelle, D. O. *Icarus* **48,** 308-328 (1981).
18. Wetherill, G. W. *Phil. Trans. R. Soc.* A**323,** 323-337 (1987).
19. Wetherill, G. W. & Shoemaker, E. M. *Geol. Soc. Am. Spec. Pap.* **190,** 1-13 (1982).
20. Palme, H., Grieve, R. A. F. & Wolf, R. *Geochim. cosmochim. Acta* **45,** 2417-2424 (1981).
21. ReVelle, D. O. J. *J. atmos. terr. Phys.* **41,** 453-473 (1979).
22. Mason, B. *Principles of Geochemistry* 3rd edn (Wiley, New York, 1966).
23. Anders, E. *Acct. chem. Res.* **1,** 289-298 (1968).
24. Turekian, K. K. & Clark, S. P. Jr. *Earth planet. Sci. Lett.* **6,** 346-348 (1969).
25. Anders, E. & Owen, T. *Science* **198,** 453-465 (1977).
26. Matsui, T. & Abe, Y. *Nature* **322,** 526-528 (1986).
27. Zahnle, K. J., Kasting, J. F. & Pollack, J. B. *Icarus* **74,** 62-97 (1988).
28. Hayatsu, R. & Anders, E. *Topics curr. Chem.* **99,** 1-37 (1981).
29. Gradie, J. & Tedesco, E. *Science* **216,** 1405-1407 (1982).
30. Morgan, J. W. *J. geophys. Res.* **91,** 12375-12387 (1986).
31. Kimura, K., Lewis, R. S. & Anders, E. *Geochim. cosmochim. Acta* **38,** 683-701 (1974).
32. Morgan, J. W., Wandless, G. A., Petrie, R. K. & Irving, A. *J. Tectonophys.* **75,** 47-67 (1981).
33. Chyba, C. F. *Nature* **330,** 632-635 (1987).
34. Ip, W.-H. & Fernandez, J. A. *Icarus* **74,** 47-61 (1988).
35. Gros, J., Takahashi, H., Hertogen, J., Morgan, J. W. & Anders, E. *Proc. Lunar Sci. Conf.* **7,** 2403-2435 (1976).
36. Hertogen, J., Janssens M.-J., Takahashi, H., Palme, H. & Anders, E. *Proc Lunar Sci. Conf.* **8,** 17-45 (1977).
37. Chapman, C. R. & Davis, D. R. *Science* **190,** 553-556 (1975).
38. Stribling, S. & Miller, S. L. *Origins Life* **17,** 261-273 (1987).
39. Deamer, D. W. *Nature* **317,** 792-794 (1985).
40. Mullie, F. & Reisse, J. *Topics curr. Chem.* **139,** 83-117 (1987).
41. Cronin, J. R., Pizzarello, S. & Cruikshank, D. P. in *Meteorites and the Early Solar System* (eds Kerridge, J. F. & Matthews, M. S.) 819-857 (Univ. Arizona Press, 1988).
42. Thomas, P. J., Chyba, C. F., Brookshaw, L. & Sagan, C. *Lunar planet. Sci.* **20,** 1117-1118 (1989).
43. Öpik, E. J. in *Proc. Geophys. Lab. Lawrence Radiation Lab. Cratering Symp.*, Rep. UCRL-6438, **2,** Paper S. 1-28 (Lawrence Radiation Lab., Berkeley, 1961).
44. Zhao, M. & Bada, J. L. *Nature* **339,** 463-465 (1989).
45. Wolbach, W. S., Lewis, R. S. & Anders, E. *Science* **230,** 167-170 (1985).
46. Lewis, R. S. & Wolbach, W. S. *Meteoritics* **21,** 434-435 (1986).
47. Hudson, B., Flynn, G. J., Fraundorf, P., Hohenberg, C. M. & Shirck, J. *Science* **211,** 383-386 (1981).
48. Emiliani, C., Kraus, E. B. & Shoemaker, E. M. *Earth planet. Sci. Lett.* **55,** 317-334 (1981).
49. Turco, R. P. *et al. Icarus* **50,** 1-52 (1982).

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4. Lang, N. D. *Phys. Rev.* **B34**, 5947-5950 (1986).
5. Korobtsov, V. V., Lifshits, V. G. & Zotov, V. G. *Surf. Sci.* **195**, 466-474 (1988).
6. Hirayama, H., Tatsumi, T. & Aizaki, N. *Surf. Sci.* **193**, L47-L52 (1988).
7. Takayanagi, K., Tanishiro, Y., Takahashi, M. & Takahashi, S. *J. Vac. Sci. Technol.* **A3**, 1502-1506 (1985).
8. Bedrossian, P. *et al. Phys. Rev. Lett.* **63**, 1257-1260 (1989).
9. Headrick, R. L., Robinson, I. K., Vlieg, E. & Feldman, L. C. *Phys. Rev. Lett.* **63**, 1253-1256 (1989).
10. Lyo, I.-W., Kaxiras, E. & Avouris, P. *Phys. Rev. Lett.* **63**, 1261-1264 (1989).
11. Feenstra, R., Stroscio, J. & Fein, A. *Surf. Sci.* **181**, 295-306 (1987).
12. Northrup, J. *Phys. Rev. Lett.* **57**, 154-157 (1986).
13. Nicholls, J. & Reihl, B. *Phys. Rev.* **B36**, 8071-8074 (1987).
14. Hamers, R. & Koch, R. in *The Physics and Chemistry of SiO_2 and the Si-SiO_2 Interface* (eds Helms, C. & Deal, B.) 201-210 (Plenum, New York, 1988).
15. Welland, M. & Koch, R. *Appl. Phys. Lett.* **48**, 724-726 (1986).
16. Petit, C. *Mosaic* **20**, 24-35 (1989).

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Determination of 4-connected framework crystal structures by simulated annealing

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THE initial derivation of atomic-scale models of the 4-connected framework crystal structures of zeolites and related materials is generally difficult. Interpretation of powder X-ray diffraction data to yield a unit cell and some symmetry information is often straightforward. Chemical analyses and sorption experiments indicate the number of framework tetrahedra, n_T, present in the unit cell (T represents the framework tetrahedral species, such as Si or Al) and the approximate pore dimensions. However, because synthetic zeolites are almost invariably microcrystalline, the possibilities for structure solution by conventional diffraction methods are limited, and framework structure determinations have traditionally relied heavily on the building of physical models. We describe here an alternative approach, in which approximate T-atom coordinates are derived from the unit-cell size and symmetry, and the value of n_T from computer modelling. An initially arbitrary T-atom configuration is optimized with respect to a 'cost function' based on the T–T distances, T–T–T angles and number of first-neighbour T-atoms, by simulated annealing using Monte Carlo methods. The potential of the method for structural determinations in both two dimensions (for structural projections) and three dimensions are illustrated by results obtained for a hexagonal cell of space group $P6/mmm$ and dimensions $a = 18.4$ Å and $c = 7.5$ Å, and by determination of the previously unknown structure of lithium gallosilicate.

A large proportion of the ~65 known zeolite framework structure types[1] were solved based on an initial determination of the 4-connected T-atom framework by, for example, physical model building[2], by consideration of direct derivatives of known structures[3], or by interpretation of electron microscopy and diffraction data[4,5]. Oxygen atoms can be added at the midpoints of each of the T–T vectors, and the T- and O-atom coordinates then optimized readily by distance least-squares (DLS) based on established constraints on the T–O, O–O and T–T distances[6,7]. The problem of structure solution reduces to one of defining approximate T-atom coordinates. The present approach describes the reasonableness of a given arrangment of the required number of T-atoms in the known unit cell in terms of a cost or 'energy' function and uses simulated annealing to adjust the T-atom coordinates to minimize that energy.

We define the total energy of a given T-atom configuration as the sum over the unique T-atoms of terms based on the T–T distances, T–T–T angles, the number, N_1, of first neighbours, and (when T-atoms are possibly located on symmetry elements) the number of symmetry operations that reproduce the original T-atom (Fig. 1). The forms of the energy as a function of the T–T distances and of the T–T–T angles are derived from histograms of observed data from a representative number of known zeolite structure types. The neighbour term is calculated from the number of T-atoms within a defined shell. The merging contribution is calculated from the distance between symmetry-related T-atoms found at less than a defined separation; merged atoms are excluded from the calculations of the other energy terms. Merging is permitted provided the total number of T-atoms per unit cell is $\geq n_T$. Appropriate relative weights of the four types of constraint were derived largely by trial and error, with the parameters being treated as tunable in complicated cases. Appropriate adjustment of these various terms (particularly the neighbour term) permits the same method to be applied to other coordination environments such as those that occur, for example, in mixed octahedral-tetrahedral framework structures.

Optimization of the coordinates of the unique T-atoms (the full unit-cell T-atom complement is generated by application of the space-group operators) to minimize the calculated energy sum is achieved by Monte Carlo methods using simulated annealing[8,9] based on an initially random configuration. During annealing low-energy, 4-connected configurations generated are stored for further evaluation (typically, 2-20 are produced in each run). Simple representations (projections down the three principal crystallographic directions) of the full unit-cell contents for each of the saved models are examined visually, followed, where appropriate, by further molecular graphics analyses, or by addition of framework oxygen atoms and DLS optimization and/or simulation of the powder-X-ray diffraction pattern(s). A typical run for a three-dimensional case (see below) with $n_{\text{unique}} = 2$ unique T-sites, 24 symmetry operators and $n_T = 36$ consumes ~60 min CPU time on a VAX8350, or ~60 s on a Cray XMP/14 se.

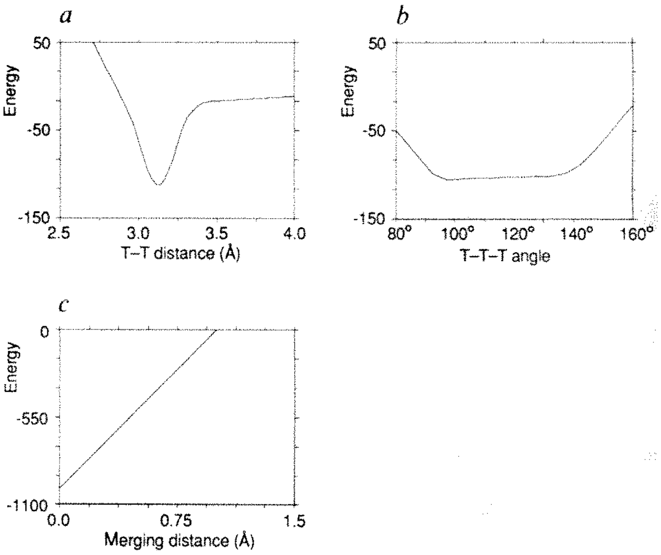

FIG. 1 Energy as a function of the T–T distance (*a*) and the T–T–T angle (*b*) used in the simulation procedure (calculated as smoothing spline fits to Boltzman equilibrium interpretations of histogrammed data taken from 32 representative zeolite crystal structures). Only the central portions are shown, the energy curves extrapolating linearly to energies of 1,000.0 and 0.0 at T–T distances of 0.0 and 5.0 Å, and to energies of 5,000.0 and 0.0 at T–T–T angles of 0.0 and 180° respectively. (*c*) The contribution to the energy sum for the merging of two symmetry-related atoms; merging is only permitted when the two atoms are at less than a defined minimum distance. The contribution to the energy sum from the coordination term is typically set to 1,000.0, 800.0, 600.0, 300.0, 0.0, 300.0, 600.0 for 0, 1, 2, 3, 4, 5 and 6 T-atoms within the defined first-neighbour radius (typically 3.7 Å) respectively.

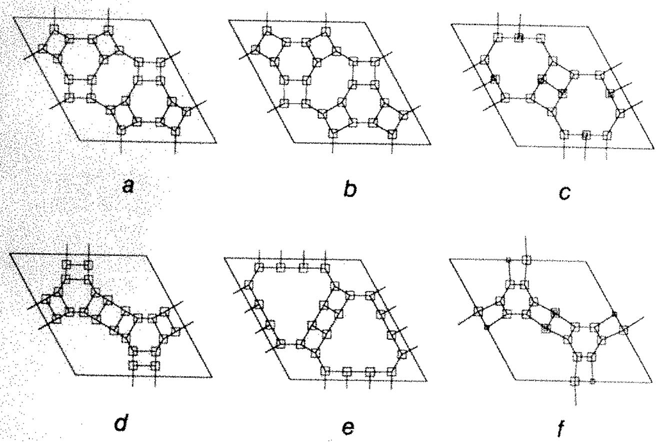

FIG. 2 The projection of the T-atom configuration in zeolite L (*a*) compared with two-dimensional modelling results (*b*–*f*) based on a hexagonal plane cell, *p6mm*, with $a = 18.4$ Å (see text). Smaller squares indicate degenerate atoms.

In the known zeolite framework structure types[1], the main pores generally run parallel to a unit-cell axis, and in projection the T-atoms are reasonably dispersed and usually 3-connected. The present methods were, therefore, developed initially based on the (simpler) two-dimensional, projection case. The constraints on both T–T distances and T–T–T angles were relaxed substantially to enable proper treatment of T–T linkages oblique to the projection direction. Results for a plane cell with *p6mm* symmetry (with 12 symmetry operators) and $a = 18.4$ Å, for $n_{\mathrm{unique}} = 2$ and $n_{\mathrm{T}} = 24$, and for $n_{\mathrm{unique}} = 2$ and $n_{\mathrm{T}} = 18$, are shown in Fig. 2. A single run with $n_{\mathrm{unique}} = 2$ and $n_{\mathrm{T}} = 24$ yielded the *ltl* net (ref. 10) (Fig. 2*b*) expected for projections of the LTL and MAZ frameworks[1], and nets labelled *tsv* (Fig. 2*d*) and *twy* (Fig. 2*e*) by Smith[10] (the projections of the three-dimensional 4-connected nets numbered 313 (ref. 11) or 81(2) (ref. 12), and 318 (ref. 11), respectively). The two-dimensional nets labelled *eoo* (ref. 10) (Fig. 2*f*)(the projection of the 81(1) or 520 net (ref. 13) adopted by the 18-ring aluminophosphates VPI-5 (ref. 14) and $ALPO_4$-54 (ref. 13) and *tfn* (ref. 10) (Fig. 2*c*) were produced in a run based on $n_{\mathrm{unique}} = 2$ and $n_{\mathrm{T}} = 18$. Similarly successful results (in a series of possible hexagonal plane symmetries) were obtained for a smaller cell with $a = 13.3$ Å that is typical of materials in the ABC-6 family of zeolites[15]. The projection approximation, however, does not converge successfully in cases such as the SOD-framework[1], where connected and nonconnected vertices have, in projection, similar separations.

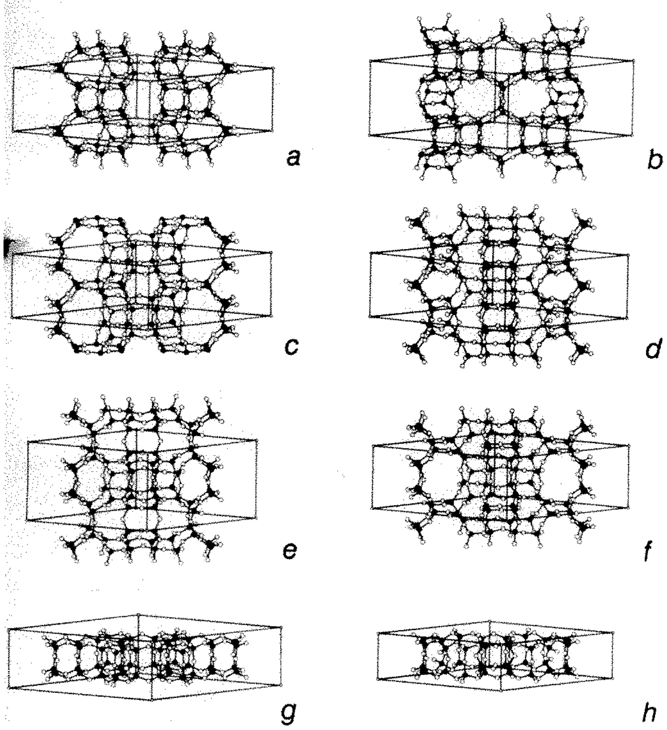

FIG. 3 Ball and stick representations of the eight distinct structure types produced in three-dimensions based on a hexagonal unit cell, *P6/mmm*, with $a = 18.4$ Å, $c = 7.5$ Å, $n_{\mathrm{unique}} = 2$ and $n_{\mathrm{T}} = 36$ that is observed for zeolite L (see text). The structures are shown following addition of the framework oxygen atoms and optimization of the atomic coordinates and the unit-cell dimensions by conventional distance least squares[6,7] (T-atoms, solid; O-atoms, open).

The full three-dimensional case is of greater utility but involves a larger number of independent variables. Annealing parameters that have been adjusted to facilitate convergence include the temperature decrement between successive cooling stages (generally chosen based on the evolution with temperature of the system specific heat[8]), and the temperature dependence of the maximum T-atom displacement in the Monte Carlo jiggle step. Typical results are illustrated in Fig. 3 for a hexagonal cell with *P6/mmm* symmetry (24 symmetry operators), $a = 18.4$ Å, $c = 7.5$ Å, and with $n_{\mathrm{unique}} = 2$ and $n_{\mathrm{T}} = 36$ that is observed for zeolite L. A total of ten different runs generated, in aggregate but with much duplication, the LTL-framework[1] (Fig. 3*a*), the hypothetical framework initially proposed for zeolite omega[16] (Fig. 3*c*), frameworks corresponding to nets numbered 313 (Fig. 3*b*), 318 (Fig. 3*d*) and 315 (Fig. 3*e*) described previously[11], a related framework with a circuit symbol $(4^3 6^3)_1(4^3 6^3)_2$ (Fig. 3*f*), and two sheet structures (Fig. 3*g* that has $n_{\mathrm{T}} = 48$, and Fig. 3*h*) that were observed with relatively high energies only at high simulation temperatures. These topologies correspond in each case to one of the two-dimensional nets deduced in the projection approximation (Figs 3*a*, 3*c* and 3*g* correspond to Fig. 2*b*; Fig. 3*b* to Fig. 2*f*, Figs 3*d* and 3*f* to Fig. 2*d*; Figs 3*e* and 3*h* to Fig. 2*e*). An example of an apparently more constrained system is provided by the high silica, 10-ring zeolite Theta-1 for which a single run (that consumed 15 min Cray XMP/14 se CPU time) based on the orthorhombic space group symmetry $Cmc2_1$, with $a = 13.8$ Å, $b = 17.4$ Å, $c = 5.0$ Å, $n_{\mathrm{unique}} = 4$ and $n_{\mathrm{T}} = 24$ converged to a single 4-connected topology identical to that described for the TON-framework in 1984[17].

Analysis of data for a condensed lithium gallosilicate that was recently described to be orthorhombic, space group *Pnam* or $Pna2_1$, with $a = 10.04$ Å, $b = 6.63$ Å, $c = 4.96$ Å (ref. 18) illustrates the solution of an unknown structure by the present method. Two runs based on this unit cell, space group $Pna2_1$, $n_{\mathrm{unique}} = 2$ and $n_{\mathrm{T}} = 8$, produced some 20 trial structures, which on inspection were found to represent different settings of three distinct 4-connected topologies. The correct model (Fig. 4) was identified by comparing the observed powder X-ray diffraction pattern with those simulated on the basis of the three models

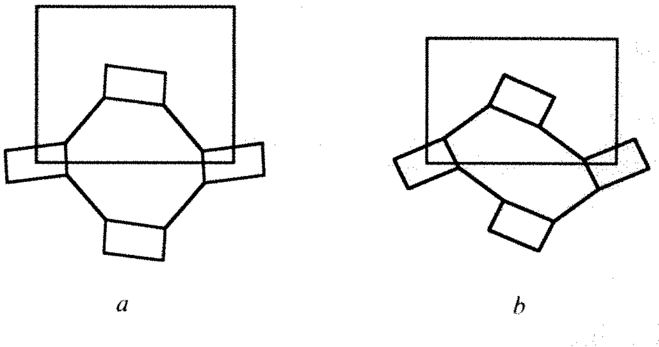

FIG. 4 The [001] projections of the framework structures of gallosilicate zeolite Li-A(BW) (*a*) compared with the DLS-optimized framework structure of its calcination product (*b*) solved by the present method.

with oxygen atoms entered (approximately) at the midpoints of the T-T vectors. This structure proves to be isotopological with gallosilicate zeolite Li-A(BW) of which it is a calcination product[18]. Removal of the sorbed water results in gross readjustment of the 8-ring channel, a 20% reduction in the length of the *b*-axis (Fig. 4), and a powder X-ray diffraction pattern that bears no resemblance to that of the parent. Although no sorption capacity for water was noted in this material[18], reversion to the parent hydrated structure may occur over extended periods under suitable water vapour pressures.

The present algorithms apparently work well for small numbers of unique T-atoms, $n_{\text{unique}} < {\sim}6$ (which encompasses >85% of the known zeolite structure types in their highest possible symmetries[1]), where a small number of passes through the program generally yields the sought solution(s). For larger numbers of independent T-atoms, especially when merging is required, many 4-connected topologies are produced. The present energy function is apparently not yet sufficiently restrictive in these more complicated cases. By evaluating results for a larger number of known materials, however, it is hoped to further improve the constraint functions and the parameters controlling passage through the simulated annealing process. Although certainly not guaranteed to work in all cases, these procedures provide a new approach to the difficult process of zeolite structure solution, and a rapid means of extending studies of hypothetical 4-connected nets. ☐

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1. Meier, W. M. & Olson, D. H. *Atlas of Zeolite Structure Types* (Butterworths, Surrey, 1987).
2. LaPierre, R. B. *et al. Zeolites* **5**, 346-348 (1985).
3. Bennett, J. M., Richardson, J. W., Pluth, J. J. & Smith, J. V. *Zeolites* **7**, 160-162 (1987).
4. Treacy, M. M. J. & Newsam, J. M. *Nature* **332**, 249-251 (1988).
5. Newsam, J. M., Treacy, M. M. J., Koetsier, W. T. & deGruyter, C. B. *Proc. R. Soc. Lond.* A**420**, 375-405 (1988).
6. Meier, W. M. & Villiger, H. *Z. Kristallogr.* **129**, 411-423 (1969).
7. Baerlocher, Ch., Hepp, A. & Meier, W. M. *DLS-76 Manual* (ETH, Zurich, 1977).
8. K. Binder (ed.) *Applications of the Monte Carlo Method in Statistical Physics* 2nd edn (Springer, Berlin, 1987).
9. Kirkpatrick, S., Gelatt, C. D. & Vecchi, M. P. *Science* **220**, 671-680 (1983).
10. Smith, J. V. in *Zeolites: Facts, Figures, Future* (eds van Santen, R. A. & Jacobs, P. A.) 29-47 (Elsevier, Amsterdam, 1989).
11. Smith, J. V. & Dytrych, W. J. *Z. Kristallogr.* **175**, 31-36 (1986).
12. Smith, J. V. & Dytrych, W. J. *Nature* **309**, 607-608 (1984).
13. Richardson, J. W., Smith, J. V. & Pluth, J. J. *J. phys. Chem.* (in the press).
14. Davis, M. E., Saldarriaga, C., Montes, C., Garces, J. & Crowder, C. *Nature* **331**, 698-699 (1988).
15. Smith, J. V. & Bennett, J. M. *Am. Miner.* **66**, 777-788 (1981).
16. Barrer, R. M. & Villiger, H. *J. chem. Soc. chem. Commun.*, 659-660 (1969).
17. Barri, S. A. I., Smith, G. W., White, D. & Young, D. *Nature* **312**, 533-534 (1984).
18. Newsam, J. M. *J. phys. Chem.* **92**, 445-452 (1987).

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Strontium, neodymium and lead isotope constraints on near-ridge seamount production beneath the South Atlantic

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STUDIES[1-7] of seamounts near the East Pacific Rise (EPR) have shown that, although most seamount lavas are petrographically and chemically identical to mid-ocean-ridge basalt, they are chemically and isotopically more diverse than those erupted on the rise axis. They are also generally more primitive (higher MgO content) and, in some cases, more depleted in incompatible elements than the axial basalts. This indicates that although near-ridge seamounts and the EPR share a common mantle source, there must be significant differences in their magma-supply processes. Seamounts are probably built by small batches of melt that rise rapidly to the surface, preserving evidence of heterogeneity in the mantle source region. By contrast, the processes of melting and melt segregation, storage and extrusion along the fast-spreading (9–13 cm yr^{-1}) EPR are more efficient in mixing and homogenizing basalt compositions[1-7]. Here we show that the relationship of strontium, neodymium and lead isotope data between seamounts in the South Atlantic and the nearby axis of the slow-spreading Mid-Atlantic Ridge (MAR) is similar to that seen in the Pacific. This indicates that the processes leading to formation of near-ridge seamounts are similar at a wide range of spreading rates. Differences in the specific isotope signatures of lavas from near-ridge seamounts and axes of the EPR and MAR reflect regional differences in the upper-mantle source of mid-ocean-ridge basalts.

The new Sr, Nd and Pb isotope data presented here (Table 1) are mostly from glassy basalts that were dredged from seamounts near the MAR during cruise RC 2802 (RV *Conrad*) in 1987[8,9] (Fig. 1). For comparison, we also present a new graphical representation of isotope data for fresh glassy basalts dredged at an average spacing of ~7 km along the MAR from the Rio Grande (25°40′ S) to the Moore (26°30′ S) fracture zones. This segment of the MAR at 26° S is spreading slowly (3-4 cm yr^{-1}) and is far from active hotspot volcanism. Indeed, all lavas from the MAR at 26° S and near-ridge seamounts are 'normal' mid-ocean-ridge basalts (MORBs) in terms of major and incompatible element contents[8,9]. As in the EPR, seamount basalts are systematically richer in MgO and more depleted in TiO_2 contents than most of the axial lavas. Major-element data indicate that the seamount basalts and some high-MgO axial lavas from near the fracture zones and the axial high (Fig. 1) were produced by smaller extents of partial melting at slightly higher pressure than most of the axial lavas[8,9].

Seamounts basalts have more variable $^{87}Sr/^{86}Sr$ and $^{143}Nd/^{144}Nd$ values than the axial lavas in the South Atlantic (Table 1 and Fig. 2*a*). Moreover, Sr isotopic ratios are generally higher and Nd isotopic ratios are lower for seamount basalts than for axial lavas. For example, samples 9 and 10, which are from one seamount, range from 0.70260 to 0.70350 in $^{87}Sr/^{86}Sr$ and from 0.51297 to 0.51314 in $^{143}Nd/^{144}Nd$ values. Sr and Nd isotopic ratios of samples 5, 6 and 7, also from a single seamount and related cones, are less variable but these ratios still are more diverse than those of axial lavas from the MAR nearby that vary from only 0.70247 to 0.70259 in $^{87}Sr/^{86}Sr$ and from 0.51308 to 0.51314 in $^{143}Nd/^{144}Nd$ values (P. C., unpublished data).

Except for sample 9, $^{206}Pb/^{204}Pb$ values of seamount basalts (18.21-18.33) are well within the range of the axial lavas (18.16–18.40) (Table 1 and Fig. 2*b*). In turn, the Pb isotope signature of the axial lavas is very similar to that of other normal-MORBs from the 22° S to 31° S segment of the MAR[10]. Seamount basalts, however, have distinctly more variable and systematically higher $^{208}Pb/^{204}Pb$ and, to a lesser extent, $^{207}Pb/^{204}Pb$, for given $^{206}Pb/^{204}Pb$ values than the axial lavas (37.86-38.17 against 37.73-38.07, and 15.47-15.55 against 15.48-15.53, respectively). Sample 9 has high $^{207}Pb/^{204}Pb$ (15.66) but approximately normal $^{208}Pb/^{204}Pb$ (38.48) for a given $^{206}Pb/^{204}Pb$ value relative to axial lavas (Fig. 2*b*).

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TABLE 1 Sr, Nd, and Pb isotopic ratios and Sr and Nd contents of representative seamount and axial lavas

Sample	$^{87}Sr/^{86}Sr$	$^{143}Nd/^{144}Nd$	$^{206}Pb/^{204}Pb$	$^{207}Pb/^{204}Pb$	$^{208}Pb/^{204}Pb$
1-1	0 702595	0 513093 (9)	18 244 (21)	15 471 (21)	37 860 (49)
1-2wr	0 702553	0 513105 (22)			
5-5	0 703629	0 512919 (10)	18 269 (31)	15 548 (27)	38 173 (68)
6-3	0 703098	0 512973 (9)	18 334 (19)	15 523 (18)	38 152 (44)
6-4	0 703155	0 512954 (9)	18 269 (12)	15 535 (11)	38 143 (24)
7-4	0 703430	0 512988 (16)			
7-5	0 703475	0 512985 (13)	18 253 (12)	15 515 (11)	38 092 (24)
9	0 703496	0 512972 (9)	18 953 (0)	15 659 (0)	38 475 (1)
9dup			18 974 (1)	15 686 (1)	38 567 (2)
10-6wr	0 702646	0 513135 (17)			
10-7wr	0 702604	0 513107 (21)	18 208 (6)	15 483 (5)	37 826 (7)
12-29	0 702552	0 513086 (23)	18 304 (3)	15 512 (2)	37 960 (7)
15-2	0 702471	0 513129 (14)	18 126 (2)	15 482 (2)	37 731 (3)
18-3	0 702525	0 513120 (17)	18 397 (2)	15 531 (2)	38 068 (7)
20-1	0 702538	0 513087 (19)	18 262 (3)	15 483 (2)	37 858 (6)

Sr isotopic ratios are fractionation corrected to $^{86}Sr/^{88}Sr=0\ 1194$ and normalized to $^{87}Sr/^{86}Sr=0\ 710265$ for NBS 987, Nd isotopic ratios are fractionation corrected to $^{146}NdO/^{144}NdO=0\ 72225$ ($^{146}Nd/^{144}Nd=0\ 7219$) and are reported relative to $^{143}Nd/^{144}Nd=0\ 511860$ for the La Jolla Standard Isotopic composition of Pb are corrected for mass fractionation based on average measured values for NBS 981 using the values given by Catanzaro *et al*[30] All analyses are done on fresh glasses except for 3 samples (indicated by wr) which are done on leached whole-rock powders Sample 9dup is an analysis on a different glass piece of sample 9 Numbers in parentheses are in-run analytical precisions and refer to the last significant digits, in-run precision for Sr isotope measurements <10

These results indicate that despite large differences in spreading rate, both the Atlantic and Pacific display similar axis-seamount isotope systematics, seamounts are more diverse isotopically than axial lavas As the abundances, morphology, and major-element contents of MAR near-axis seamounts are also similar to their Pacific counterparts[8 9], it seems likely that near-axis seamounts are produced by similar processes operating at a wide range of spreading rates Because of the gross petrological similarity of lavas from seamounts and nearby axis, the close proximity of seamounts to the axis, and because the zone of mantle upwelling is thought to be ~100 km wide[11,12], it is very clear that axial and seamount melts tap the same mantle source However, the systematic difference between axial and seamount lavas in major elements, trace elements and isotopes indicates that this common source is tapped in different ways and/or the melt migration and/or the storage history of seamount magmas and axial magmas are different

As already mentioned, the petrological diversity but generally high MgO contents of near-ridge seamount lavas indicate that seamounts tap relatively small volumes of mantle, which rise quickly without extensive modifications[1-7] In addition, seamount magmas may be the result of smaller extents of partial melting, in which case magmas may contain high proportions of more enriched, more easily melted components of the upper mantle[1,5,12] than axial lavas By contrast, axial magmas may be mixed and homogenized during melt segregation, melt migration or storage in crustal magma chambers

Despite the similarity in axis-seamount systematics in the Atlantic and Pacific there are also significant differences For example, Atlantic near-ridge seamounts show much more variability in $^{87}Sr/^{86}Sr$ than do the Pacific ones (Fig 2*a*) Atlantic seamounts exhibit smaller ranges of $^{143}Nd/^{144}Nd$ and $^{206}Pb/^{204}Pb$ variation than Pacific ones, but this could be due to the small data base for Atlantic near-ridge seamounts Note also that Atlantic seamount samples are all normal MORBs whereas in the Pacific, most of the high $^{87}Sr/^{86}Sr$ and low $^{143}Nd/^{144}Nd$ samples are alkali basalts and their differentiates[7] The reason for this is not entirely clear, but it could be due to differences in the depth of origin or age of eruption of Atlantic and Pacific near-axis seamounts Alternatively, the geochemical characteristics beneath the South Atlantic and the Pacific might be different[13]

Another notable feature of the data is that except for some lavas from the Lamont seamount chain in the Pacific[6], Atlantic near-axis seamounts have lower mean $^{206}Pb/^{204}Pb$ than Pacific ones (Fig 2*b*) The Lamont samples, however, have low $^{87}Sr/^{86}Sr$, whereas the low $^{206}Pb/^{204}Pb$ Atlantic samples have high $^{87}Sr/^{86}Sr$ (Fig 3*b*) The overall pattern of lower $^{206}Pb/^{204}Pb$ and lower $^{143}Nd/^{144}Nd$, but higher $^{207}Pb/^{204}Pb$ and $^{208}Pb/^{204}Pb$ for a given $^{206}Pb/^{204}Pb$ of Atlantic seamounts relative to Pacific ones (Figs 2*b*, 3*a*) is apparently paralleled by a similar isotope pattern at the respective ridge axes The MAR in the South Atlantic has a lower $^{206}Pb/^{204}Pb$ than the EPR (Fig 2*b*) Like-

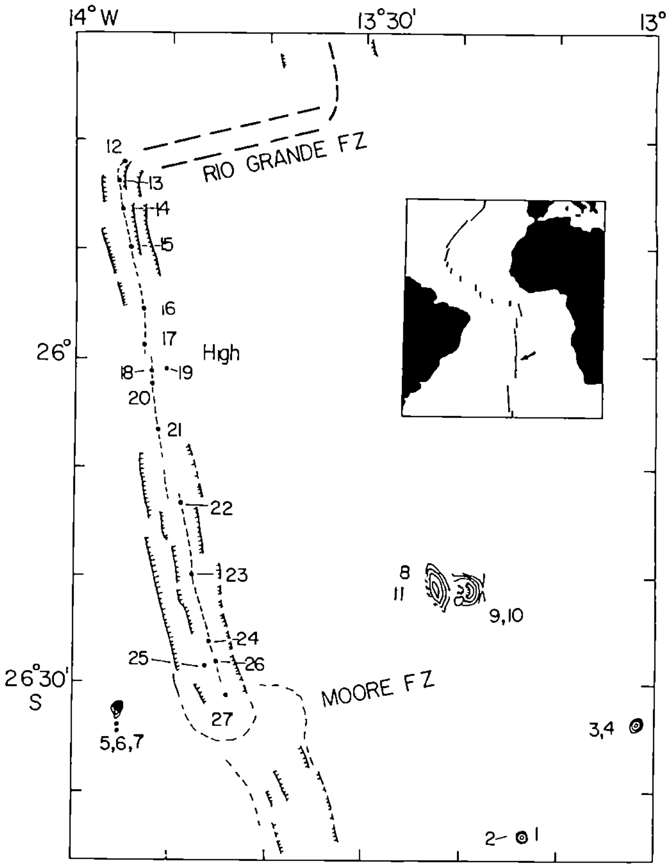

FIG 1 Schematic map of the Mid-Atlantic Ridge between the Rio-Grande and Moore fracture zones, showing dredges on near-ridge seamounts (1-11), along the MAR axis (12-18, 20-24, 26, 27) and slightly off-axis (19, 25) (from ref 8) The ridge axis is elevated near the centre of the segment at ~26° S as indicated

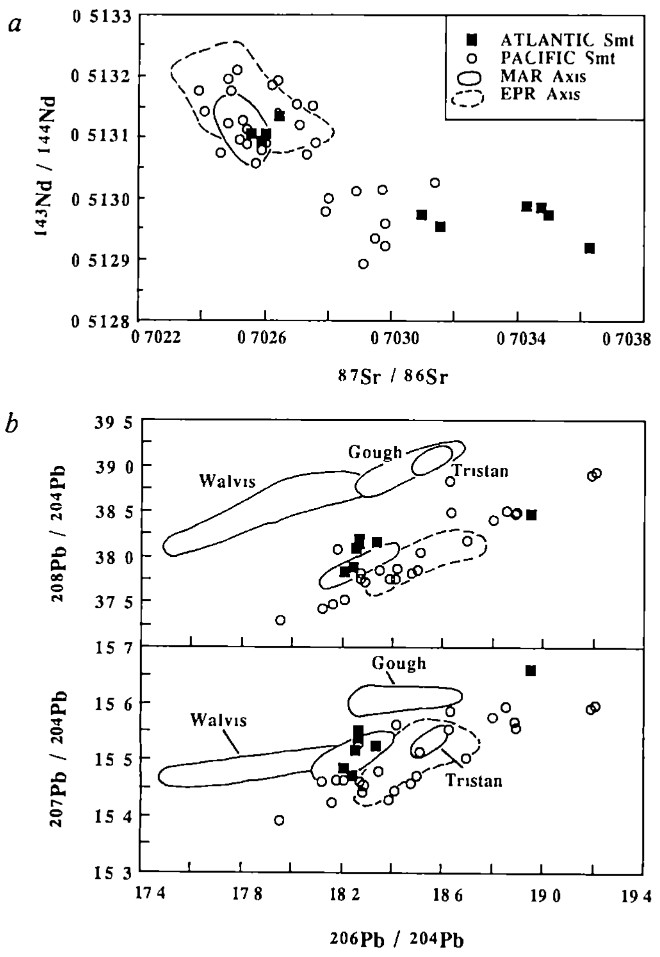

FIG 2 *a*, Plot of ^{143}Nd/^{144}Nd against ^{87}Sr/^{86}Sr for lavas from near-ridge seamounts in the Atlantic and Pacific Isotope data for axial lavas shown for reference are restricted to those segments of the MAR and EPR that are adjacent to the seamounts These isotope data are from the MAR between 25° 40′ and 26° 30′ S (representative samples from dredges 12-27 in Fig 1) and from the EPR between 8° to 22° N latitude *b*, plots of ^{208}Pb/^{204}Pb and ^{207}Pb/^{204}Pb against ^{206}Pb/^{204}Pb for the samples shown in *a* Symbols and reference fields as in *a* (Pb isotope data for an axial lava from the MAR at 26° 00′ S reported by Hanan and Schilling[10] (EN063 12D-5g) plot inside the field for MAR axis) Note that although almost all lavas from near-ridge seamounts and the ridge axis in the south Atlantic have similar ^{206}Pb/^{204}Pb, seamount basalts have higher ^{208}Pb/^{204}Pb and ^{207}Pb/^{204}Pb than axial lavas This Pb isotopic signature is a common feature of lavas from the Tristan da Cunha and Gough hot spots and their apparent trace, the Walvis Ridge, further to the south Data from near-ridge seamounts in the Pacific with lowest ^{206}Pb/^{204}Pb values are from the Lamont group of seamounts[6] Despite the fewer number of available samples from the South Atlantic, near-ridge seamounts clearly are more isotopically heterogeneous than axial lavas

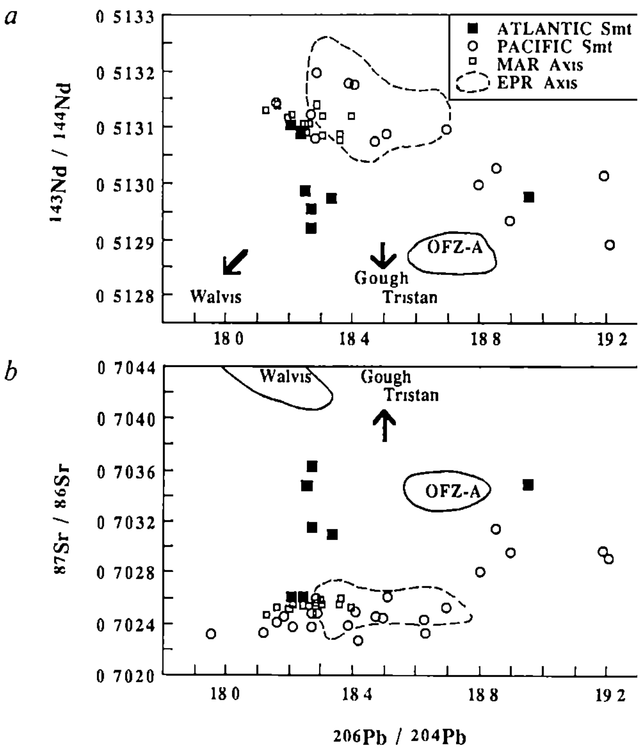

FIG 3 Plots of ^{143}Nd/^{144}Nd against ^{206}Pb/^{204}Pb (*a*) and ^{87}Sr/^{86}Sr against ^{206}Pb/^{204}Pb (*b*) for lavas from near-ridge seamounts in the Atlantic and Pacific Axial lavas from the MAR at 26° S generally have lower mean values of ^{206}Pb/^{204}Pb and lower mean values of ^{143}Nd/^{144}Nd but higher mean values of ^{207}Pb/^{204}Pb and ^{208}Pb/^{204}Pb for given ^{206}Pb/^{204}Pb than axial lavas from the EPR between 8°-22° N These regional isotope differences generally are more pronounced in the isotopic signature of lavas from near-ridge seamounts Nd isotopic ratios of the Lamont seamount basalts are not available, nevertheless, the fairly good negative correlation between Nd and Sr isotopic ratios of other seamounts near the EPR[5,7] indicate that these lavas also have lower ^{143}Nd/^{144}Nd for given ^{206}Pb/^{204}Pb ratios than Atlantic seamount and axial lavas Fields for the Tristan da Cunha, Gough, Walvis Ridge, and 'type A' Oceanographer Fracture Zone lavas[13] are shown for reference Note that all near-ridge seamount and axial isotope data can be enclosed by a single large triangle (not shown) in these two diagrams

wise, the MAR in the South Atlantic has a lower mean ^{143}Nd/^{144}Nd but higher mean ^{207}Pb/^{204}Pb and ^{208}Pb/^{204}Pb than the EPR (Fig 2*b*) These observations are consistent with the notion of a similar mantle source for both axial and seamount magmas

With the exception of some samples from near the Oceanographer Fracture Zone[14] and near the Equator[15], the MAR in the North Atlantic exhibits an isotope pattern different from that in the South Atlantic[10,13] For example, North Atlantic axial MORBs far from hotspots generally have similar ^{206}Pb/^{204}Pb and higher ^{143}Nd/^{144}Nd than the EPR axial lavas[16,17] These apparent differences between the MAR in the North and South Atlantic may be due to regional differences in the mantle characteristics[13], but a possible plume contribution in the South Atlantic[10] is difficult to rule out

The comparison between the South MAR and EPR indicates that near-axis seamount lavas tend to magnify isotope differences that are present but more subdued in axial lavas In the southern MAR, our new data confirm the presence of at least three mantle components as proposed by Hanan *et al*[10] Two of these are also present at the EPR and the third is a component enriched in the Tristan-Gough-Discovery hotspots (Fig 3*a*,*b*) The presence of this component in the near-ridge seamount and in the MAR axial lavas is difficult to explain The large geographic distance between the MAR at 26° S and the hotspots (31°-47° S) is inconsistent with present models of hotspot-ridge interaction[10,18,19] Moreover, this component is also recognized in MORBs from the North Atlantic[14,15] and elsewhere in the Indian Ocean[20,21] posing further difficulty for a simple hotspot contamination model Our preferred hypothesis[13,22] is that the component is pervasive in areas underlain by low-velocity, presumably hot, regions in the mantle[23] recognized by tomographic studies[24] These regions also are correlated with hotspot concentration and geoid anomalies[25,26] One such region is below the southern tip of Africa but extends north to the Azores[13,23]

New isotope data also add further evidence that the suggested inverse relationship between spreading rate and isotope variability[27,28] is not always valid Our results as well of those of others[16,17,29] show that apparently there is no such relationship Rather, both the fast-spreading EPR and the slow-spreading MAR are isotopically variable, the observed isotope difference is most probably due to regional differences of heterogeneous mantle components beneath spreading centres ☐

1 Batiza R & Vanko D *J geophys Res* **26**, 564-602 (1984)
2 Allan J F Batiza R & Lonsdale P *Seamounts Islands and Atolls Geophys Monogr* No 43 (eds Keating B H *et al*) 255-282 (Am geophys Un Washington DC 1987)
3 Allan J F Batiza R Perfit M R Fornari D J & Sack R O *J Petrol* (submitted)
4 Fornari D J Perfit M R Allan J F & Batiza R *Nature* **331** 511-513 (1988)
5 Zindler A Studigel H & Batiza R *Earth planet Sci Lett* **70**, 175-195 (1984)
6 Fornari D J *et al Earth planet Sci Lett.* **89**, 63-83 (1988)
7 Graham D W *et al Contr Miner Petr* **99**, 446-463 (1988)
8 Batiza R Melson W G & O Hearn T *Nature* **335**, 428-431 (1988)
9 Batiza R *et al J Geol* **97**, 209-220 (1989)
10 Hanan B B Kingsley R H & Schilling J-G *Nature* **322**, 137-144 (1986)
11 McKenzie D P *Earth planet Sci Lett.* **74**, 81-91 (1985)
12 Sleep N H *J geophys Res* **89**, 10029-10041 (1984)
13 Castillo P *Nature* **336**, 667-670 (1988)
14 Shirey S Bender J F & Langmuir C H *Nature* **325**, 217-223 (1987)
15 Schilling J-G Hanan B B & McCully B *Eos* **69**, 1426 (1988)
16 White W M Hofmann A W & Puchelt H *J geophys Res* **92**, 4881 4893 (1987)
17 Ito E White W M & Gopel C *Chem Geol* **62**, 157-176 (1987)
18 Schilling J-G *Earth planet Sci Lett* **25**, 103-115 (1975)
19 Schilling J G *Nature* **314**, 62-67 (1985)
20 Klein E M Langmuir C Zindler A Staudigel H & Hamelin B *Nature* **333**, 623-529 (1988)
21 Mahoney J J *et al J geophys Res* **94**, 4033-4052 (1989)
22 Castillo P *Eos* **69**, 490 (1988)
23 Silver P G Carlson R W & Olson P A *A Rev Earth planet. Sci* **16**, 477-541 (1988)
24 Dziewonski A M *J geophys Res* **89**, 5929-5952 (1984)
25 Hager B H *J geophys Res* **89**, 6003-6015 (1984)
26 Cazenave A Souriau A & Dominh K *Nature* **340**, 54-57 (1989)
27 Cohen R S & O Nions R K *J Petrol* **23**, 299-239 (1982)
28 Allègre C J Hamelin B & Dupre B *Earth planet Sci Lett.* **71**, 71-84 (1984)
29 Macdougal J D & Lugmair G W *Earth planet Sci Lett.* **77**, 273-284 (1986)
30 Catanzaro E J Murphy T J Shields W R & Garner E L *J Res natn Bur Stand* **72**A (1968)

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Young high-temperature granulites from the base of the crust in central Mexico

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CHARACTERIZATION of the lower continental crust is essential to understanding processes of crustal formation and evolution Many workers have concluded that the lower crust is composed of granulite-facies rocks (see, for example, refs 1, 2), but many exposed granulite-facies terranes seem to have mid-crustal rather than lower-crustal origins[3-5] Here we present new data from direct samples of the lower continental crust, in the form of metapelitic rock fragments brought to the surface by Quaternary volcanism in central Mexico These xenoliths contain exsolved ternary feldspars that record extremely high minimum metamorphic temperatures of 950-1,100 °C, the highest yet known to be preserved in deep-seated metamorphic rocks The metamorphism seems to be the result of underplating by basaltic magma and to be regional in extent Our barometry on these samples demonstrates that granulite-facies metamorphic rocks are present in the lower crust, and provides important constraints on tectonic models and interpretations of geophysical data. Chemical and textural evidence suggest that the xenoliths were not able to cool to below 850-900 °C before being entrained in their host magmas, indicating that the granulite-facies metamorphism occurred no more than ~30 Myr ago

So far most studies of xenoliths have focused on characterization of samples from the Earth's upper mantle[6], and only limited mineralogical and geochemical data have been obtained on xenoliths believed to be from the lower crust In addition, most studies of deep crustal xenoliths have concentrated on mafic and intermediate orthogneisses[6] Although modally less abundant than mafic orthogneisses, xenoliths with sedimentary protoliths may be more useful in providing definitive pressure information, because their mineralogy is often more appropriate for applying existing geobarometers[7-10]

Xenoliths of garnet-sillimanite paragneiss, occurring in Quaternary basanitic tuffs[11] from the central Mexican plateau in the state of San Luis Potosí, contain the assemblage garnet-sillimanite-mesoperthite-quartz-rutile-graphite, ±plagioclase-ilmenite-zircon-monazite The paragneisses are the major lithology at El Toro and represent ~10% of the xenolith population at La Joya Honda and Los Palau, mafic and felsic orthogneisses are the dominant lithologies at these two localities Here we present the pressure (P)-temperature (T) conditions of last equilibration for the paragneisses, and discuss the crustal level from which these samples were derived and the information they reveal about the crust beneath central Mexico

The paragneiss xenoliths were selected for detailed study based on mineralogical and textural similarities and the presence of univariant mineral assemblages with respect to barometric reactions Five samples are from the El Toro (ET) cinder cone, three are from La Joya Honda (LJH) maar, and two are from Los Palau (LP) maar Field relations are given elsewhere[11-13] The xenoliths range from 4 to 10 cm in diameter and appear fresh In general, all samples have similar mineral chemistries None of the selected samples exhibit distinct layering or mineral zoning Evidence of partial fusion is given by the ubiquitous occurrence of devitrified melt rims around garnets (see, for example, refs 14, 15) Quench textures in the melt rims consist of intergrowths of euhedral orthopyroxene, hercynite-spinel$_{ss}$, and interstitial glass and/or calcic plagioclase Stahle[14] has suggested that the breakdown of almandine garnet to orthopyroxene+hercynite+glass occurs rapidly at ~1,100 °C and 0 1-0 3 GPa

The compositions of the feldspars may provide constraints on the thermal histories of the xenoliths Plagioclase lamellae occurring within an alkali feldspar host and large plagioclase crystals not hosted by an alkali feldspar have very similar anorthite contents, within a given sample The plagioclase lamellae are typically slightly more potassic, indicating that the larger plagioclase crystals formed during cooling from an initially homogeneous alkali feldspar[16] Exsolution lamellae in feldspars are typically 10-30 µm in width Exsolved feldspar compositions are given in Fig 1*a* and Tables 1 and 2 The calculated

TABLE 1 Representative host, lamellae and reintegrated feldspar analyses from a single mesoperthite crystal in sample ET24

	Kfs host	Pl lam	Reintegrated		
SiO_2	64 15	58 98	60 64	60 85	61 22
Al_2O_3	19 51	25 53	22 98	23 33	23 47
Fe_2O_3	0 01	0 19	ND*	ND*	ND*
CaO	0 77	7 41	4 30	4 42	4 81
Na_2O	3 12	6 42	5 21	5 24	5 30
K_2O	11 60	1 56	5 82	5 45	5 23
Total	99 16	100 09	98 95	99 29	100 03
Formulae normalized to 5 cations					
Si	2 94	2 63	2 76	2 76	2 76
Al	1 06	1 35	1 23	1 25	1 25
Fe^{3+}	0 00	0 01	ND*	ND*	ND*
Ca	0 04	0 36	0 21	0 21	0 23
Na	0 28	0 56	0 46	0 46	0 46
K	0 68	0 09	0 34	0 32	0 30
O	7 99	7 98	7 97	7 99	8 00

* Fe_2O_3 was not determined (ND) for the reintegrated analyses
Kfs alkali-feldspar, Pl lam plagioclase lamella

TABLE 2 Component mole percentages for each sample from averaged mineral analyses

Sample number	Garnet Al	Garnet Py	Garnet Gr	Rutile TiO_2	Ilmenite $FeTiO_3$	Fs host An	Fs host Or	Fs lam An	Fs lam Or	Integ An	Integ Or	Temperature (°C) Int	Temperature (°C) Exs
ET11	55	39	5	98	95	4	73	39	10	20	40	1,100	880
ET16	52	43	4	98	—	3	69	34	10	10	54	1,000	890
										23*	30*	1,050	890
ET24	49	46	4	98	98	4	69	35	9	22	32	1,050	880
ET38	55	40	4	97	—	3	73	36	10	23	33	1,075	880
ET42	55	40	4	97	—	4	65	30	12	18	34	1,025	890
LJH14	49	45	4	97	—	3	66	32	14	15	44	1,050	910
LJH15	54	42	3	98	—	6	52	27	17	19	28	1,000	965
LJH32	53	43	3	98	89	6	51	26	15	19	26	975	940
LP7	54	42	3	98	84	5	59	33	14	23	30	1,050	950
LP8	50	45	3	97	81	4	56	23	24	9	49	950	950

Sample locality abbreviations are given in the text

Mineral abbreviations Al, almandine, Py, pyrope, Gr, grossular, An, anorthite, Or, orthoclase, Fs, feldspar, Lam, lamellae Spessartine and albite can be calculated by difference from 100 Int, average minimum reintegrated temperature, and Exs, average temperature of exsolution using the model of Lindsley and Nekvasil[17]

* Bimodal distribution of reintegrated analyses observed for this sample

tie lines for 900 °C from the ternary feldspar model of Lindsley and Nekvasil[17] are shown at $P = 1\ 0$ GPa, the pressure dependence of the feldspar equilibria is from ref 18 The mesoperthites contain lamellar plagioclase ($An_{20-40}Ab_{49-62}Or_{8-28}$) and alkali feldspar ($An_{3-7}Ab_{20-45}Or_{47-76}$) (Tables 1 and 2) Graphical use of the ternary two-feldspar thermometer in ref 17 yields temperatures of ~800–950 °C for lamellae and host feldspars (Fig 1*a* and Table 2) These temperatures are much higher than any recorded by exsolved feldspars of exposed granulites[16,19] Reintegration of the mesoperthites by image analysis and broad-beam energy-dispersive analysis yields former ternary feldspar compositions of $An_{6-24}Ab_{35-57}Or_{22-55}$ (Tables 1 and 2) The compositions are among the most ternary reported from rocks of undoubted metamorphic origin[16,20-23] The isotherms of the ref 17 model require minimum temperatures of 950–1,125 °C (Table 2 and Fig 1*b*) for the reintegrated feldspars to be stable

Tie lines connecting exsolved alkali feldspar and plagioclase compositions for El Toro samples agree well with calculated tie lines[17] (Fig 1*a*) La Joya Honda and Los Palau samples give mixed results for exsolved compositions, as tie lines cross, indicating that these compositions may not represent equilibrium assemblages Nevertheless, the reintegrated values give similar minimum temperatures of 950–1,125 °C for the three localities Regardless of the details of exsolution processes, these mesoperthites yield minimum reintegrated temperatures that are considerably higher than those typically obtained for exposed granulites[16,19]

Figure 2 is a back-scattered electron image of a mesoperthite from sample ET24, quartz and rutile are also present The regular and continuous exsolution texture is typical of the mesoperthites, and is interpreted as evidence for slow cooling after regional metamorphism If the mesoperthites were produced during or after transport to the surface it is highly unlikely that the intergrowths would be so coarse and regular The homogeneity of lamellae and host compositions within each xenolith suggests that equilibrium between the feldspars was commonly attained, and no chemical gradients across lamellae have been identified with the electron microprobe The occurrence of mesoperthite inclusions in unzoned garnets from three samples (LP7, LP8, LJH32) seems to exclude an origin by epitaxial growth, as proposed[23] for mesoperthites from Kilbourne Hole (New Mexico), and indicates that the garnets

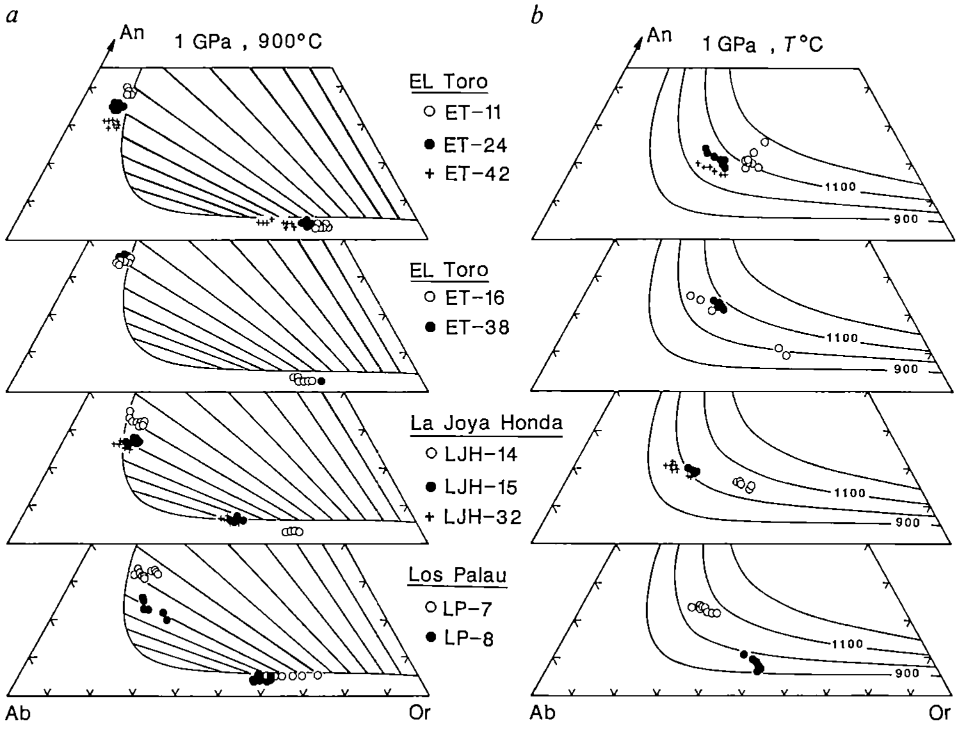

FIG 1 *a*, Exsolved feldspar compositions, for each xenolith, plotted on stacked anorthite (An)–albite (Ab)–orthoclase (Or) diagrams Symbols correspond to sample numbers as shown Isotherm and tie lines for 900 °C are shown at 1 0 GPa (ref 17) *b*, Reintegrated feldspar compositions plotted as in *a* Isotherms are shown at 1 0 GPa for 900, 1,000, 1,100 and 1,200 °C (ref 17) equilibrated with homogeneous ternary feldspars. Diffusion through the garnet would be required to permit re-equilibration of exsolved plagioclase compositions with matrix sillimanite.

The paragneiss garnets are homogeneous, with almandine ranging from 49 to 55 mol %, pyrope from 39 to 47 mol % and grossular from 3 to 5 mol % (Table 2). Garnets contain abundant inclusions of quartz, sillimanite and rutile. If the garnet in each sample equilibrated with the reintegrated ternary feldspar, sillimanite ($X_{Al_2SiO_5} = 0.99$), rutile ($X_{TiO_2} = 0.97$-0.98), ilmenite ($X_{FeTiO_3} = 0.81$-0.98) and quartz, barometry may be applied using the feldspar activity/composition relations of ref. 17. For garnet we have used the mixing model of Anovitz and Essene[24]. An ideal-solution model was used for rutile, sillimanite and ilmenite, and the activity of quartz was assumed to be unity. The garnet-sillimanite-quartz-plagioclase barometer[8] yields pressures of 0.9-1.4 GPa at $T = 950$-$1{,}200$ °C; the garnet-rutile-ilmenite-plagioclase-quartz barometer[9] also gives pressures of ~0.9-1.3 GPa. If lower pressure limits from the five samples that lack ilmenite are excluded, pressures of 0.9-1.3 GPa are obtained with the garnet-rutile-sillimanite-ilmenite-quartz barometer[10]. Pressures deduced from the three barometers correspond to depths of ~33-48 km (depending on temperature and the average specific gravity of the crustal rock column, chosen here to be 2.7). These depths are consistent with the estimated crustal thickness (35-45 km) in the study area[25-27], and show that the xenoliths sample the region of the crust/mantle boundary in central Mexico.

The mesoperthites provide evidence for regional metamorphism at $T > 950$-$1{,}125$ °C, higher than any temperatures recorded previously in regionally metamorphosed rocks of undoubted sedimentary parentage. The metamorphism spanned an area of at least 100 km^2 in central Mexico and may be related to the occurrence of similar metapelitic xenoliths 1,600 km to the north at Kilbourne Hole in New Mexico in the United States. We conclude that the lowermost crust of central Mexico was heated to ~1,100 °C by underplated basaltic magmas[5,28]. The base of the crust was subsequently cooled to ~900 °C, as recorded by the exsolved feldspars. We believe the xenoliths never equilibrated below 900 °C and were excavated from nearly the depth they record by ascending basanitic magmas. Such extreme quench temperatures (800-950 °C) for the feldspars suggest recent granulite facies metamorphism of the base of the crust in central Mexico; a mid-Tertiary underplating event would be consistent with moderate cooling rates of 5-10 °C Myr^{-1} for a regional terrane. These inferences are consistent with data in a chronological study of Kilbourne Hole metapelites, in which a metamorphic age of 1.6 Gyr was obtained from Rb-Sr isochrons of adjacent layers and an age of 34 (±14) Myr from mineral isochrons[29].

We have attempted to unravel the cooling history of the xenoliths using data on the coarsening of exsolution lamellae in alkali feldspars[30], and the homogenization of plagioclases[31]. Data from coarsening experiments[30] indicate that lamellae of width 10-30 μm could be produced in 10^5 yr at 800 °C, and 10^{-3} yr at 1,100 °C (with an initial lamella width of 0 μm), but calculated homogenization rates[31] are orders of magnitude slower. At 1,100 °C, lamellae 10 μm wide would be homogenized in $10^{4\pm1.5}$ yr, and $\sim10^{10\pm1}$ yr are needed for 10-μm-wide lamellae at 800 °C. Although the homogenization rates, for which experiments involved coupled NaSi-CaAl diffusion[31], are consistent with the preservation of feldspar lamellae in the xenoliths, the coarsening estimates are in sharp disagreement with our hypothesis of slow cooling subsequent to a mid-Tertiary regional metamorphic event. Perhaps the strongest evidence against a mesoperthite origin related to the host basalt is the absence of coarse perthites in volcanic rocks; to our knowledge all volcanic perthite occurrences are cryptoperthites/microperthites with lamellae that are ≤1 μm wide. We believe that available experiments on lamellar coarsening are not relevant to ternary mesoperthites because the experiments were done on cryptoperthites with coherent structures at a pressure of 10^5 Pa (1 atm) and at temperatures <600 °C, and, most importantly, they were done on binary alkali feldspars. Although higher temperatures should enhance coarsening, coupled Al/Si diffusion, required for exsolution of ternary feldspars, will greatly hinder coarsening[30-32]. As the Mexican mesoperthites exsolved on the strain-free solvus with non-coherent structures, a mechanism involving nucleation and growth is required—a slower process than spinodal decomposition. Coarsening rates calculated from existing experimental data are inconsistent with

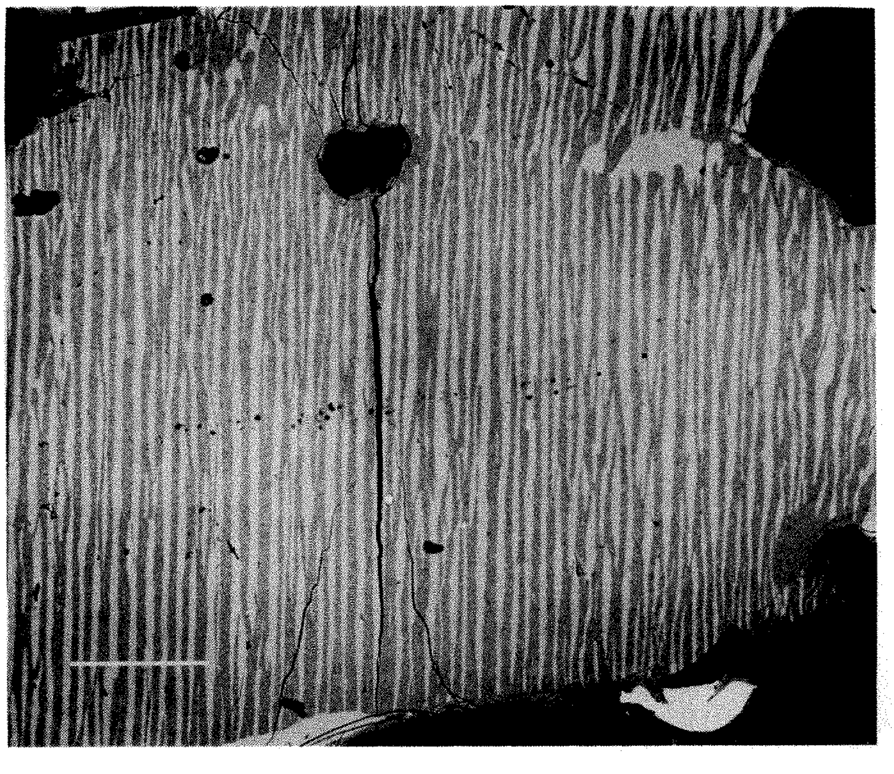

FIG. 2 Back-scattered electron image of a typical mesoperthite (sample ET24) showing the regular and continuous nature of exsolved plagioclase lamellae (darker bands). Quartz (dark) and rutile (light) are also present. Scale bar = 150 μm.

TABLE 1 Coefficients of relatedness (r) for *Mischocyttarus* and *Polistes* females

	Collection		Sample sizes			
Species	Locality	Date	colonies	females	loci	$r \pm$ s.e.
M. basimacula	Yucatan	Dec 1987	16	75	2	0.435 ± 0.116
M. immarginatus	Yucatan	Dec 1987	19	123	3	0.765 ± 0.036
P. versicolor	Venezuela	July 1988	18	107	4	0.371 ± 0.084
P. canadensis	Venezuela	July 1988	20	129	3	0.339 ± 0.097
P. annularis	Texas	Oct 1986	34	156	2	0.306 ± 0.075
P. instabilis	Texas	Oct 1986	42	227	2	0.525 ± 0.064
P. exclamans 1	South Texas	Oct 1986	46	312	1	0.557 ± 0.085
P. exclamans 2	East Texas	Oct 1986	33	277	3	0.692 ± 0.104
P. metricus	Texas	Oct 1986	19	145	5	0.567 ± 0.087
P. carolinus	Texas	Aug 1984	16	59	6	0.630 ± 0.059
P. dorsalis	Texas	Oct 1986	21	204	4	0.607 ± 0.079
P. bellicosus	Texas	Oct 1986	31	289	1	0.338 ± 0.148
P. dominulus	Italy	Sept 1988	8	57	4	0.652 ± 0.088
P. gallicus	Italy	Sept 1988	12	134	2	0.803 ± 0.150
P. nimpha	Italy	Sept 1988	10	103	3	0.540 ± 0.162

Sampling for each species was generally restricted to small (<6 ha) areas. When this was not true, data were combined from several small areas when an F_{ST} close to zero indicated homgeneity, or treated separately when there was significant differentiation (*P. exclamans*).

of some species will overwinter and serve as helpers to one of their natal nestmates who was not the queen of the natal nest[13,14,20], so their relatedness to the brood they help rear is only half of their relatedness to the nestmate who becomes the queen.

When relatedness to colony mates is less than $\frac{1}{2}$, there are two possible explanations for the selective maintenance of eusociality. The first is that individuals may be able to direct their aid towards colony mates who are especially closely related, perhaps rearing full sisters even when most colony mates are half-sisters. Such discrimination has been demonstrated in honeybees[21,22], though the effect is generally weak. It requires that individuals use cues learned from their own phenotypes. This seems unlikely for *Polistes* because adults isolated from their nest subsequently fail to distinguish kin from non-kin[23]. These experiments suggest that kin-recognition cues are derived from the nest or brood, sources that are unlikely to carry information useful for within-colony discriminations. Thus, the available evidence seems to support the second possible explanation, which is that reproductive altruism may evolve even when $r < \frac{1}{2}$. According to inclusive fitness theory[1,2], helping is favoured when $rb > \frac{1}{2}c$, where b is the fitness benefit to the individual helped, c is the cost to the helper, r is relatedness to the brood reared, and $\frac{1}{2}$ is the relatedness of the helper to its own offspring. So even when $r < \frac{1}{2}$, altruism can be favoured when the benefit is sufficiently higher than the cost. Several studies have shown that benefits may exceed costs in *Polistes*[24-27].

Although the data do not support the prediction that individuals usually gain a relatedness advantage by rearing offspring that are not their own, they do confirm some of Hamilton's ideas[1,2]. With the addition of our 14 species to the 3 previously studied primitively eusocial species[9-12], there are sufficient data to support the generalization that relatedness is always fairly high in species without morphological castes. This permits us to draw an interesting contrast to species with castes. In five species of social wasps with at least some degree of caste development, relatedness takes on lower values, ranging from 0.11 to 0.40 (refs 19, 28). For ants with highly specilized worker castes, relatedness within colonies is variable, but is extremely low in some species[29-31]. Thus when individuals are constrained by a specialized worker morphology they may accept a subordinate, nonreproductive role to aid individuals that may not be highly related. However, as expected from inclusive fitness theory, to the extent that individuals are unconstrained in their choices, they perform such sacrifices only for rather close relatives. □

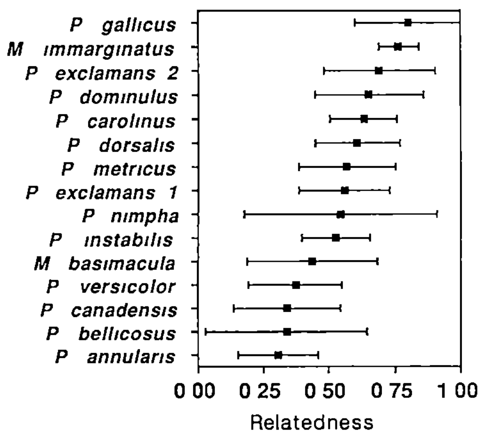

FIG. 1 Point estimates and 95% confidence intervals for relatedness among female nestmates in *Polistes* and *Mischocyttarus*. Confidence intervals are calculated using values of the t-distribution with degrees of freedom equal to one less than the number of colonies.

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1 Hamilton W D *J theor Biol* **7**, 1-52 (1964)
2 Hamilton W D *A Rev Ecol Syste* **3**, 193-232 (1972)
3 Trivers R L & Hare H *Science* **191**, 249-263 (1976)
4 Craig R & Crozier R H *Evolution* **33**, 335-341 (1979)
5 Pamilo P & Varvio-Aho S-L *Behav Ecol Sociobiol* **6**, 91-98 (1979)
6 Pamilo P & Crozier R H *Theor Popul Biol* **21**, 171-193 (1982)
7 Pamilo P *Genetics* **107**, 307-320 (1984)
8 Queller D C & Goodnight K F *Evolution* **43**, 258-275 (1989)
9 Crozier R H Smith B H & Crozier Y C *Evolution* **41**, 902-910 (1987)
10 Kukuk P F in *Genetics of Social Evolution* (eds Breed M D & Page R E) 183-202 (Westview Boulder 1989)
11 Schwarz M P *Behav Ecol Sociobiol* **21**, 387-392 (1987)
12 Ross K G & Matthews R W *Amer Nat* (in the press)
13 Pardi L *Boll Ist Entom Univ Bologna* **15**, 25-84 (1942)
14 West-Eberhard M J *Misc Publ Mus Zool Univ Mich* **140**, 1-101 (1969)
15 Litte M *Behav Ecol Sociobiol* **2**, 229-246 (1977)
16 Strassmann J E *Z Tierpsychol* **69**, 141-148 (1985)
17 Metcalf R A & Whitt G S *Behav Ecol Sociobiol* **2** 339-351 (1977)
18 Lester L J & Selander R K *Amer Nat* **117**, 147-176 (1981)
19 Queller D C Strassmann J E & Hughes C R *Science* **242**, 1155-1157 (1988)
20 Strassmann J E *Science* **204**, 207-209 (1979)
21 Frumhoff P C & Schneider S *Anim Behav* **35**, 255-262 (1986)
22 Page R E Robinson G E & Fondrk M K *Nature* **338**, 576-579 (1989)
23 Gamboa G J Reeve H K & Pfennig D W *A Rev Entomol* **31**, 431-454 (1986)
24 Metcalf R A & Whitt G S *Behav Ecol Sociobiol* **2**, 353-360 (1977)
25 Noonan K M in *Natural Selection and Social Behavior* (eds Alexander R D & Tinkle D W) 18-44 (Chiron New York 1981)
26 Strassmann J E Queller D C & Hughes C R *Ecology* **69**, 1497-1505 (1988)
27 Queller D C *Proc natn Acad Sci USA* **86**, 3224-3226 (1989)
28 Ross K G *Nature* **323**, 398-400 (1986)
29 Pamilo P *Heredity* **48**, 95-106 (1982)

30 Pearson B *Behav Ecol Sociobiol* **12**, 1-4 (1983)
31 Ross K G & Fletcher D J C *Behav Ecol Sociobiol* **17**, 349-356 (1985)

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Telemetered *in vivo* strain analysis of locomotor mechanics of brachiating gibbons

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THE slender elongated form that is characteristic of the forelimb long bones of gibbons (*Hylobates*) has long been attributed to their functional adaptation to habitual armswinging locomotion[1–3], although potential selective advantages of this morphology for brachiation have yet to be demonstrated If the forces exerted on the limb skeleton during brachiation indeed differ greatly from those of other locomotor modes, then the changes in skeletal loading accompanying a shift in locomotor behaviour could favour alterations in skeletal morphology in brachiating lineages *In vivo* skeletal strain patterns recorded by using radiotelemetry during brachiation indicate that the forelimb bones of the gibbon are loaded in substantial tension and show reduced bending and compression in comparison with those of other mammals We suggest that this unique loading regime could have contributed to the evolution of the distinctive morphology of hylobatid limbs

Theoretical analyses of the mechanics of brachiation have modelled gibbon locomotion after ideal pendula[4,5], these models predict that the supporting limb is subjected to axial tension under the load exerted by gravity acting on the animal's mass Correspondingly, it has been suggested that tensile loading is the key feature distinguishing brachiation from walking, running and jumping[6,7] However, models of the response of bone to mechanical loading have argued against the possibility that bones are ever regularly subjected to net tension[8] Furthermore, because all vertebrate long bones so far studied experience primarily bending and compressive forces[9–12], axial tensile loading is a novel loading regime for a limb This would alter the criteria for natural selection acting on skeletal design, given that a structure's ability to resist tensile, but not compressive and bending forces, is independent of both length and cross-sectional shape, and that bending, particularly due to eccentric loads, increases in proportion to length[13] If, in brachiation, bone compression and bending are minimal and tension predominates, an increased bone length will not be structurally disadvantageous

Tensile loading may not characterize suspensory support, however, because skeletal loading depends not only on external forces but also on internally transmitted, largely compressive muscle forces To resolve this issue, we recorded *in vivo* surface bone strains at the dorsal and ventral midshaft of the humerus, radius and ulna of four freely brachiating white-handed gibbons (*Hylobates lar*) by using radiotelemetry[14] Principal strain magnitudes and their orientations with respect to the bone's long axis were determined for each rosette strain gauge, and used to characterize bone loading[15]

For all forelimb long bones, principal strain magnitude peaked at midsupport phase of the swing, as the animal's body reached the bottom of the arc swept out by the forelimb, near-zero strains were obtained when the limb was not grasping a support (Fig 1) Considerable variability in strain pattern and peak magnitude among swings was observed, contrasting with the stereotypic patterns recorded in walking and running animals[9,11,16] This seems to be due both to the unrestricted movement in a large enclosure compared with controlled treadmill locomotion, and to development of localized strain from muscles attaching near the gauges

The largest principal strains recorded on the dorsal and ventral surfaces of the ulna were tensile, similar in magnitude, and oriented close to the bone's longitudinal axis, indicating overall axial tension (95% of total strain) with a small amount of bending (Fig 2*a*, Table 1) In the radius, principal strains were again oriented longitudinally, and showed substantial compression on the ventral midshaft surface with smaller tensile strains dorsally (Fig 2*b*, Table 1) Unlike the ulna, loading of the radius was characterized by dorso-ventral bending (83% of total strain) superimposed on axial compression In the humerus, peak principal strains for both the dorsal and ventral surfaces were tensile but differed in magnitude, and strain orientation deviated 45-60° from the bone's axis (Fig 2*c*, Table 1) The humerus thus experienced a combination of axial tension, torsion (indicated by the off-axis orientation) and bending

The overall axial tension recorded from the ulna was similar (within 11%) to values expected for a bone functioning as a passive suspending cord of a pendulum[14] Given this tensile loading and the predicted net tensile loading of the forelimb as a whole, the bending and compression of the radius may seem surprising This loading disparity arises from differences between the two bones in morphology, transmitted muscular force, and weight-support roles In long bones, the main source of bending may be axial force exerted about the longitudinal curvature of the bone[17], in the gibbon, radii are seven times more curved than ulnae[14] Muscle force is typically the greatest constituent of axial force exerted on long bones (generally two to three times the magnitude of the reaction force of the body acting against the ground[9]) Gibbon forearm flexor muscles are positioned ventrally so that their pull will bend the bone in a ventrally concave fashion, dictated by the radius's dorso-ventral curvature, and during the support phase of brachiation, flexor activity is marked[18–20] Additionally, weight is transferred in tension directly from the humerus to the ulna through the interlocking humero-ulnar joint, whereas the direct connections from humerus to radius are exclusively ligamentous, and the interosseus membrane joining the two forearm bones is not stiff

TABLE 1 Mean peak maximum principal strain magnitudes and orientations

	$\mu\varepsilon$	(s d)	Orientation	(s d)
Ventral ulna	+1,421	(337)	2°	5°
Dorsal ulna	+1,270	(167)	6°	1°
Ventral radius	−1,638	(453)	1°	2°
Dorsal radius	+746	(248)	5°	4°
Ventral humerus	+419	(180)	−44°	3°
Dorsal humerus	+1,492	(71)	+62°	4°

Mean peak-maximum principal strain magnitudes (in microstrain, $\mu\varepsilon$) and orientations (in degrees deviation from the longitudinal axis of the bone) for each recording site Tensile strains are positive values, compressive strains negative Orientations are positive if they deviate from the bone's long axis in the proximo-medial direction, negative if they deviate in the proximo-lateral direction The s d of the values for each site are given in parentheses after the mean value or thick enough to transfer substantial stress between them Compression of the radius indicates both that muscle force is preferentially exerted through the radius rather than through the ulna, and that this force provides the primary loading of the radius, whereas the animal's body weight is transferred from the humerus to the hand through the ulna

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The lesser tensile strain recorded from the humerus compared with that recorded from the ulna probably results from compressive forces exerted by the arm and shoulder musculature[18-20] counteracting tension from suspensory weight support, and the six-times greater cross-sectional area of the humerus[14] The curvature of the humerus and the differential muscle activity on the flexor and extensor aspects of the arm probably produces the bending we observed The significant torsion of the humerus reflects the bone's resistance to the lateral rotation of the gibbon's body about the long axis of the limb during the swing[21] During this movement, the humerus rotates little at the shoulder joint and the supporting hand remains stationary[22] Thus, free rotation of the radius and proximal carpus about the humerus and distal carpus provides propulsive motion[22,23] while freeing the forearm bones from torsion Stabilization at the shoulder combined with the hinge-like morphology of the humero-ulnar joint can then result in torsional loading of the humerus

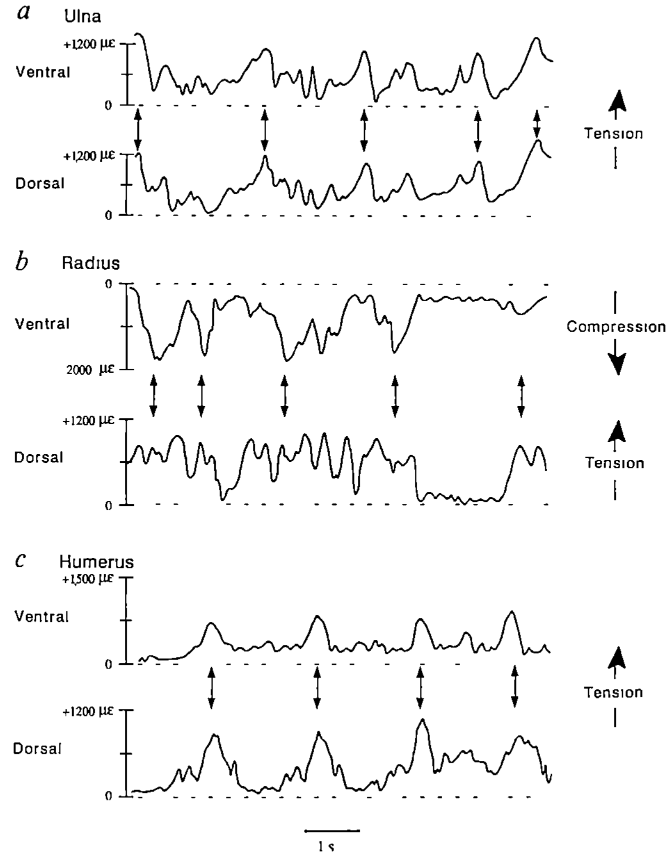

FIG 1 Representative principal strain recordings from the ventral and dorsal midshaft surfaces of the ulna (*a*), radius (*b*) and humerus (*c*) of one gibbon for a duration of 8 s, correlated with swing kinematics based on light films (60 frames per second) taken during recordings The midsupport phase of each swing is indicated by an upward pointing arrow Strain magnitudes are in microstrain (strain $\times 10^{-6}$) Tensile strains are positive and compressive strains are negative Bone strains reached their greatest absolute values at midswing, and strain values return close to zero as the limb is swung forward (protracted) during the nonsupporting phase of the locomotor cycle (analogous to the swing phase of terrestrial locomotion) All recordings were made at the Yerkes Regional Primate Research Center, Emory University, Atlanta, Georgia By using aseptic surgery under general anaesthesia, we attached rosette strain gauges with a self-catalysing cyanoacrylate adhesive to the dorsal and ventral midshaft cortices of the right humerus and left radius and ulna at the bones' diaphyses, as near as possible to midshaft level but in areas free of direct muscle attachment to minimize localized distortions in overall bone strain due to muscle pull The procedure was similar to that previously described[9,24] Lead wires were led subcutaneously up the arm and over the shoulder, externalized through the skin between the scapulae at approximately the level of the third thoracic vertebra, and soldered to a connector, all lead wires were left slack to permit rapid and forceful limb movements without dislodging the gauges Following 2–3 days post-operative recovery, *in vivo* strain data were then recorded over a 2–3 day period using radiotelemetry Each gibbon carried a strain transmitter on its back as it brachiated freely in a large (9 × 2 × 3 m) outdoor enclosure The eight bridge channels were multiplexed to a single channel telemetry transmitter (Transkinetics TXM-205, frequency response, 35 kHz, transmission range, 50 m) which was housed in the pocket of a vest-style harness that fitted snugly around each animal's chest and back Each channel was sampled at a frequency of 125 Hz After 2–3 days of recordings, all gauges and wires were removed from the animals

Our results show that some bones can be regularly loaded in net tension Hence, models of adaptive bone remodelling positing bone resorption under tensile loading must be revised Our results also indicate a new explanation for the elongation of the forelimb skeleton in gibbons that is based on the reduction of compressive and bending loads inherent in suspensory support In general, limb elongation is advantageous in terms of increased stride length, velocity and greater reach, especially in grasping primates However, greater limb-bone length is probably constrained in most terrestrial mammals partly by the risks engendered by excessive bending and the associated large strains when long slender bones experience off-axis loading In contrast to terrestrial locomotion, in which external loading (weight support) and muscle forces are additive in generating compressive

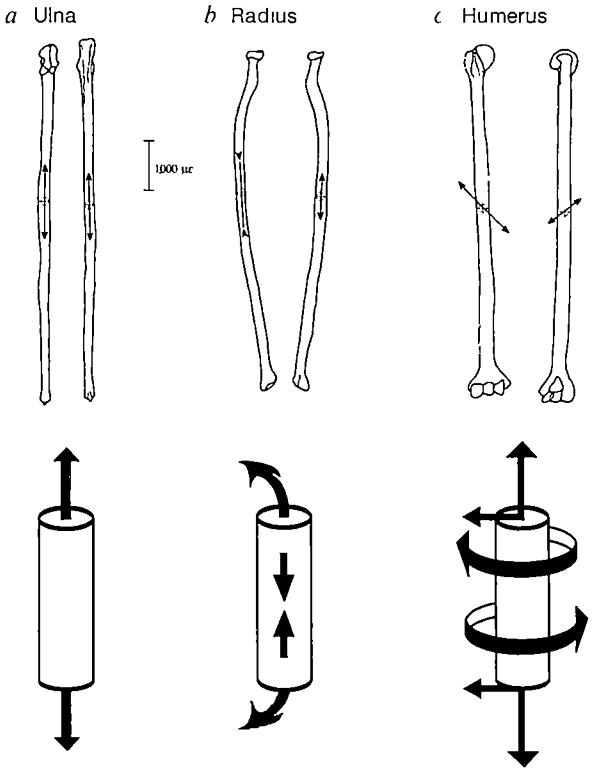

FIG 2 Diagramatic views of the ventral (left) and dorsal (right) ulna (*A*), radius (*B*) and humerus (*C*) with strain-gauge attachment sites indicated by the cross-hatched circles Vectors depicting mean peak principal-strain magnitude and orientation with respect to the bone's longitudinal axis as recorded from the implanted strain gauges are indicated at each gauge attachment site, outwardly directed arrows indicate tension, whereas inwardly directed arrows indicate compression The cylindrical model below each bone schematically shows each bone's overall loading mode ulna, axial tension, radius, compression and dorsoventral bending, and humerus, tension, dorsoventral bending and torsion

and bending stresses, muscle forces (compressive) and external weight support (tensile) will oppose one another during brachiation, reducing net axial strain in the skeleton. Brachiation further reduces bending of the forelimb skeleton by suspending the animal's weight directly in line with the bones' long axes at the time in the swing cycle when external forces are greatest[14]. By reducing bending, selection for increased bone length in brachiators could be favoured with minimal risk or increased strain and hence bone failure. ☐

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1 Keith A J Anat 37 18-40 (1903)
2 Andrews P & Groves C E in *Gibbon and Siamang* (ed Rumbaugh D M) 167-218 (Karger Basel 1976)
3 Preuschoft H & Demes B in *Size and Scaling in Primate Biology* (ed Jungers W L) 383-399 (Plenum New York 1985)
4 Fleagle J G *Nature* **248**, 259-260 (1974)
5 Preuschoft H & Demes B in *The Lesser Apes Evolutionary and Behavioral Biology* (eds Preuschoft H Chivers D J Brockelman W V & Creel N) (Edinburgh University Press 1984)
6 Kummer B *Anthrop Anz* **32**, 74-82 (1970)
7 Oxnard C E *The Order of Man* (Yale University Press New Haven 1984)
8 Currey J D *The Mechanical Adaptations of Bones* (Princeton University Press New Jersey 1984)
9 Biewener A A Thomason J Goodship A E & Lanyon L E *J Biomech* **16**, 565-576 (1983)
10 Lanyon L E & Bourne S *J Bone Jt Surg* **A61**, 263-273 (1979)
11 Biewener A A Swartz S M & Bertram J E A *Calcif Tissue Int* **39**, 390-395 (1986)
12 Biewener A A & Taylor C R *J exp Biol* **123**, 383-400 (1986)
13 Wainwright S A Biggs W D Currey J D & Gosline J M *Mechanical Design in Organisms* (Wiley New York 1976)
14 Swartz S M *The Biomechanics and Structural Design of the Forelimb of Brachiating Primates* (The University of Chicago Press 1988)
15 Dally J W & Riley W F *Experimental Stress Analysis* (McGraw-Hill 1978)
16 Rubin C T & Lanyon L E *J exp Biol* **101**, 187-212 (1982)
17 Bertram J E A & Biewener A A *J theor Biol* **131**, 75-92 (1988)
18 Jungers W L & Stern J T Jr *Science* **208**, 617-619 (1980)
19 Jungers W L & Stern J T Jr *Int J Primat* **2**, 18-33 (1981)
20 Jungers W L & Stern J T Jr in *The Lesser Apes Evolutionary and Behavioral Biology* (eds Preuschoft H Chivers D J Brockelman W V & Creel N) 119-134 (Edinburgh University Press 1984)
21 Avis V *Southw J Anthrop* **18**, 119-148 (1962)
22 Jenkins F A Jr Bombroski P J & Gordon E P *Am J phys Anthrop* **48**, 65-76 (1978)
23 Jenkins F A Jr *Symp zool Soc Lond* **48**, 429-451 (1981)
24 Lanyon L E & Baggot D G *J Bone Jt Surg* **B58**, 436-443 (1976)

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Changes in spinal cord reflexes after cross-anastomosis of cutaneous and muscle nerves in the adult rat

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EVIDENCE exists that the specification of afferent nerves and their central connections in the embryo may depend in part on influences from the peripheral target innervated[1–7]. We have now investigated whether such peripheral determination persists in the adult rat using the unmyelinated afferent system of C fibres, which differ chemically in the adult depending on their target[8–11]. We have previously shown that if the cutaneous sural nerve and the muscle gastrocnemius nerve are cross-anastomosed so that they grow to each other's target, the A fibres establish functional endings and the C fibres change their chemistry to that which is appropriate for the new target[9,12–14]. Here we report that in normal adult rats, a short train of stimuli to the cutaneous sural nerve produced a brief facilitation of the flexion reflex, lasting on average only 5 min, whereas similar stimulation of the gastrocnemius-muscle nerve enhanced this reflex for an average of 54 min. In cross-anastomosed animals, stimulation of the gastrocnemius nerve (innervating skin) induced a brief potentiation of the flexion reflex, lasting on average only 3 min. By contrast, stimulation of sural nerve (innervating muscle) produced a potentiation of this reflex lasting 57 min. Thus the ability of adult afferent nerves to potentiate the flexion reflex depends on the target with which they make contact. We propose that tissue-specific factors influence some of the central actions of primary afferent neurons in the adult.

In adult decerebrate low-spinal rats, with normal nerves or cross-anastomosed or self-anastomosed sural nerves (s n) and gastrocnemius nerves (made 10–14 weeks previously), the flexion reflex was measured as the discharge in small branches of the nerve to biceps femoris/semitendinosus containing a few motorneurons[15]. The flexion reflex was elicited by applying a standard 3-s suprathreshold pinch stimulus to the middle three toes of the ipsilateral hindpaw, and the multiunit discharge was recorded and counted for the 3 s of the stimulus. The reflex was initially similar in all animals and consisted of a phasic high-frequency discharge lasting 0.5-2 s, and its magnitude was stable from trial to trial. Repeated determinations of the flexion reflex were made at 5-min intervals. After obtaining consistent control responses over at least a 20 min period, conditioning stimuli of 20 shocks at C-fibre strength (5 mA, 500 μs, 1 Hz) were given to the normal or anastomosed nerves, and the subsequent changes in the size of the flexion reflex were determined at 1-min intervals for 5 min, and at 5-min intervals thereafter.

Conditioning the normal sural nerve increased the response to the standard pinch stimulus both in terms of the frequency of firing of motorneurons and duration for which they fired. The facilitation persisted for on average 5±1.6 min (±s e m). The magnitude of the enhancement of the flexion reflex was typically 200–300%. Figure 1 shows the time course of change in one typical case.

When the same conditioning stimulus was applied to normal g n there was an enhancement of the flexion reflex lasting for an average of 54±8.3 min. A typical example is shown in Fig 1. The difference in duration of facilitations was highly significant ($P < 0.01$), and is similar to that previously reported for normal animals[15,16]. The modulation induced by stimuli conditioning g n often appeared to have two phases: an early phase resem-

TABLE 1 Enhancement of flexion reflex induced by conditioning stimulus

Nerve stimulated	Average enhancement (min)	s e m	No
Normal s n	5	1.6	28
S n reinnervating skin	2	0.4	4
S n innervating muscle	59	9.4	5
Normal g n	54	8.3	16
G n reinnervating muscle	77	11.3	3
G n innervating skin	3	0.7	6

The duration of enhancement of flexion reflex (in min) induced by a conditioning stimulus (20 shocks, 5 mA, 500 μs, 1 Hz) delivered either to the normal s n or g n, or to s n and g n which had been cut and had reinnervated their own normal targets, or to s n and g n which had innervated inappropriate targets after cross-anastomosis.

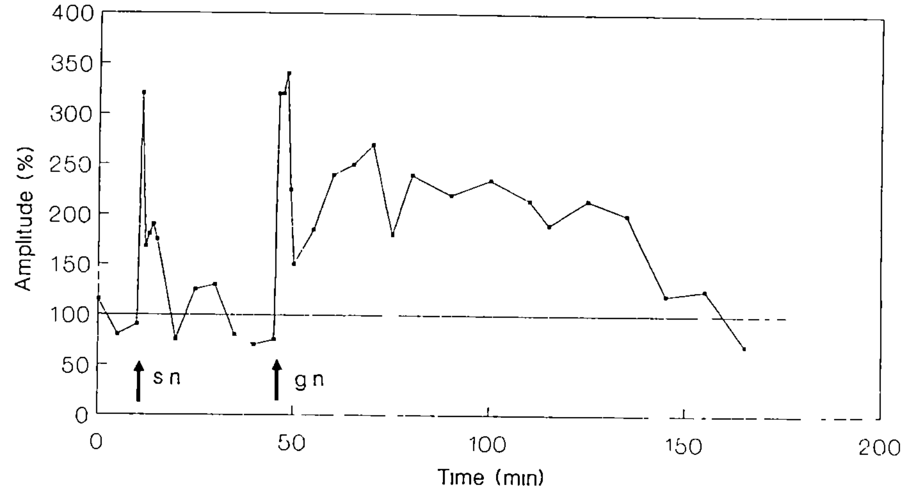

FIG 1 The time course of enhancement of the flexion reflex in one normal animal after a conditioning stimulus train to the s n (at a time indicated by arrow marked s n) and the g n (at a time indicated by arrow marked g n) The magnitude of the flexion reflex (the number of discharges evoked by the standard pinch stimulus) is plotted as a percentage of the average level determined in the control period Note that whereas the s n produced only a short-lived facilitation, the g n produced a more prolonged facilitation

METHODS A small branch of the nerve to biceps femoris was dissected, typically containing 5-10 motorneurons activated by the pinch test stimuli Multiunit efferent action potentials were recorded using conventional techniques with amplifier filters set at 1 kHz to 8 kHz, and counted with a window discriminator set to detect all voltage fluctuations greater than three times the noise level The total number of counts occurring in the 3 s after the pinch stimuli are plotted here as a percentage of the counts obtained in the control period The duration of facilitation was measured as the time taken for the amplitude of the reflex to fall below 120% of the control level

bling that seen with the conditioning of s n and a later phase which reached a maximum with a latency of 10-20 min The order of presentation of conditioning stimuli did not affect the nature of facilitations

Recordings on cross- or self-anastomosed nerves showed that the nerves had successfully innervated skin or muscle[12 13] The distal nerve trunk appeared to be of a normal size We have previously shown that >70% of peripheral fibres successfully negotiate the anastomosis and regenerate to the inappropriate target[12 13]

The conditioning stimuli produced very different changes in animals with the cross-anastomosed nerves Stimuli to the g n , then innervating skin, resulted in an enhancement of the flexion reflex, but this lasted only 3±0 7 min (compared with 54± 8 3 min in normal animals, $P<0\ 01$, Fig 2) Stimulation of s n , then innervating muscle, produced a long-lasting facilitation, starting within 1 or 2 min after application of the conditioning stimulus, and lasting 59±9 4 min (compared with 5 min in normal animals, $P<0\ 01$) These facilitations also tended to show an early and a late phase, like the effect seen in normal g n Table 1 shows the duration of the effects elicited by trains of conditioning stimuli to the different nerves

For self-anastomosed nerves, the nature of the flexion reflex and the facilitatory effects of conditioning the s n and g n were quantitatively similar to those in intact animals That is, conditioning the s n enhanced the flexion reflex for 2±0 4 min, and conditioning the g n enhanced the reflex for 77±11 3 min ($P>0\ 05$, compared with intact controls in both cases, Table 1) One typical example is shown in Fig 3 The onset of facilitation was also similar, in all cases beginning 1-3 min after the conditioning stimulus The only apparent difference between responses in intact and self-anastomosed nerves was in the magnitude of facilitation, which tended to be less in the latter group, typically 150-250% Hence, the changes seen with cross-anastomosed nerves cannot be attributed to a nonspecific consequence of nerve section or regrowth

To avoid the possibility of spread of electric current, conditioning by the injury discharge resulting from nerve section was used Wall and Woolf[16] noted that enhancement of the flexion reflex could be triggered by the injury discharge produced by section of peripheral nerves as well as by electrical stimulation In normal animals, the section of s n induced a relatively short-lived enhancement of the flexion reflex, whereas that from the section of g n was much more prolonged ($P<0\ 01$, see Table 2) Similar facilitations were seen with self-anastomosed nerves (Table 2) By contrast, in the animals with

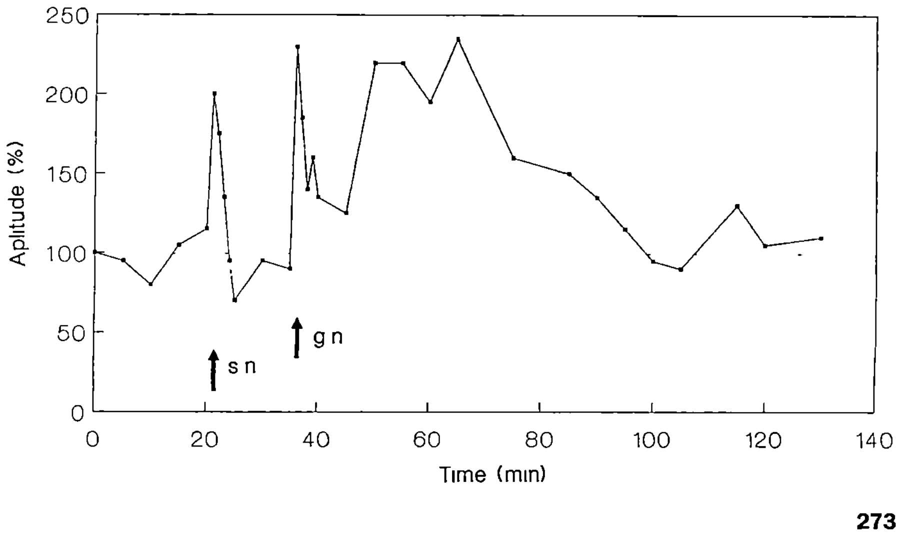

FIG 2 The time course of enhancement of the flexion reflex in one animal with cross-anastomoses of the s n and g n The figure is constructed in the same way as Fig 1 Note that in this animal, in contrast to normal animals, the s n (innervating muscle) produced a long-lasting facilitation, whereas the g n does not

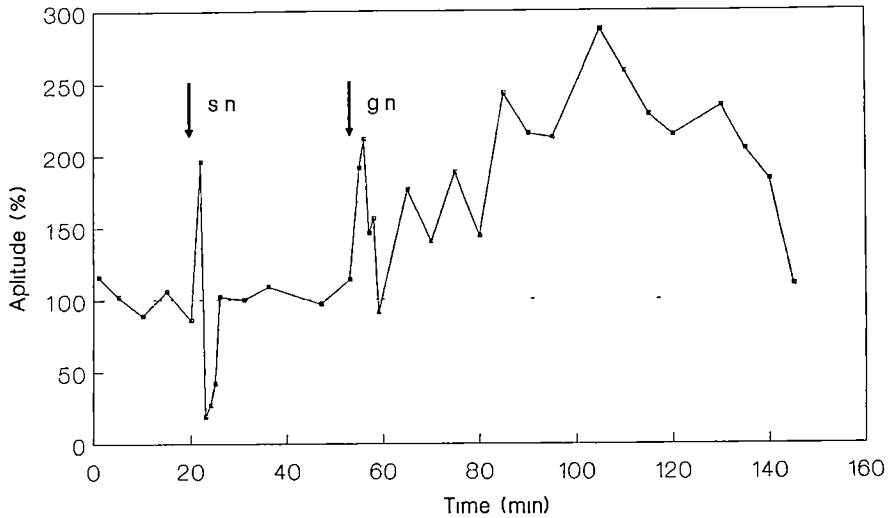

FIG 3 The time course of enhancement of the flexion reflex in one animal with self-anastomoses of the s n and g n The figure is constructed in the same way as Fig 1 Note that in this animal, like animals with intact nerves, the g n (innervating muscle) produced a long-lasting facilitation, whereas the s n (innervating skin), does not

cross-anastomoses, the section of s n (supplying muscle) elicited long-lasting effects, whereas the section of the g n (innervating skin), only short-lasting changes ($P<0$ 01, Table 2)

The facilitation of the flexion reflex is known to depend on the activation of afferent C-fibers and to be due to an altered heterosynaptic excitability within the spinal cord[16 17] When the sural or gastrocnemius nerves reinnervate inappropriate tissue they change their normal characteristics and take on those appropriate for the tissue that they then innervate That is, s n afferents innervating muscle induce long-lasting facilitation, whereas the g n afferents innervating skin lose this capacity These changes in the spinal cord are presumably secondary to alterations in the properties of primary afferents when they innervate inappropriate tissue The inappropriate tissue is presumably the skin or muscle itself, although it could be the non-neural elements in the nerves leading to the tissue We have previously shown that such inappropriate reinnervation can alter the histochemistry of primary afferents Normal s n C-fibres contain high levels of substance P and the enzyme FRAP (fluoride resistant acid phospatase), whereas normal g n afferents do not, when these nerves are cross-anastomosed, the expression of these markers become appropriate for the tissue innervated[9 14] It could be that the altered ability to modulate the flexion reflex reflects some parallel change in the histochemistry of the primary afferents

Thus, peripheral targets can not only regulate some properties of primary afferents, but can extend this influence to a control of their central effects In adult animals, isolation of afferents from their peripheral targets by axotomy can produce several plastic changes within the central nervous system, including a reorganization of the receptive fields of dorsal horn cells[18-21] The strength of monosynaptic connections from Ia fibres may be reduced when these fibres inappropriately reinnervate non-spindle targets[22] We have recently found[24] that when gastrocnemius afferents regenerate to skin, there is a dramatic change in the central responses after their stimulation In normal animals, stimulation of unmyelinated g n afferents excites relatively few dorsal-horn cells In animals with cross-anastomosed nerves, the central effect of gastrocnemius afferent barrages is greatly enhanced and many more dorsal-horn neurons are excited by these afferents This too is not a non-specific effect due to axotomy or regrowth, but is dependent on some influence arising from the new tissue innervated The changes we have reported here could similarly indicate the presence of tissue-specific trophic influences, the effects of which on primary afferents could further influence the establishment or maintenance of specific patterns of central responses ☐

TABLE 2 Enhancement of flexion reflex induced by injury discharge

Nerve stimulated	Average enhancement (min)	s e m	No
Normal s n	13	2 7	7
S n reinnervating skin	7	2 8	4
S n innervating muscle	67	8 3	4
Normal g n	64	7 5	13
G n reinnervating muscle	52	4 7	3
G n innervating skin	4	1 1	4

The duration of enhancement of flexion reflex (in min) induced by the injury discharge from axotomy of either the normal s n or g n, or of self-anastomosed or of cross-anastomosed s n and g n innervating either appropriate or inappropriate targets

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1 Barakat I & Droz B *Neuroscience* **28**, 39-47 (1989)
2 Frank E & Westerfield M *J Physiol* **324**, 495-505 (1982)
3 Marunsich M F Pourmehr K & Weston J A *Devl Biol* **118**, 505-510 (1986)
4 Smith C L & Frank E *Soc Neurosci Abs* **10**, 100 34 (1984)
5 Smith C L & Frank E *Soc Neurosci Abs* **11**, 63 (1985)
6 Smith C L & Frank E *J comp Neurol* **269**, 96-108 (1988)
7 Smith C L & Frank E *Brain Behav Evol* **31**, 227-242 (1988)
8 McMahon S B Sykova E Wall P D Woolf C J & Gibson S *Neurosci Lett* **52**, 235-240 (1984)
9 McMahon S B & Moore C E *J comp Neurol* **274**, 1-8 (1988)
10 Gibson S J *et al Peptides* **7**, 273-283 (1986)
11 Honda C N Rethelyi M & Petrusz P *J Neurosci* **3**, 2183-2196 (1983)
12 Lewin G R & McMahon S B *Pflügers Arch ges Physiol* **411**, R179 (1988)
13 Lewin G R & McMahon S B *Eur J Neurosci Sup* 151 16 (1988)
14 McMahon S B & Gibson S *Neurosci Lett* **73**, 9-15 (1987)
15 Wall P D & Woolf C J *J Physiol* **356**, 443-458 (1984)
16 Woolf C J & Wall P D *J Neurosci* **6**, 1433-1442 (1986)
17 Woolf C J *Nature* **306**, 686-688 (1983)
18 Devor M & Wall P D *Nature* **276**, 75-76 (1975)
19 Wall P D & Devor M *Brain Res* **209**, 95-111 (1981)
20 Wall P D Fitzgerald M & Gibson S J *Neurosci* **6**, 2205-2215 (1981)
21 Merzenich M M *et al Neuroscience* **8**, 33-55 (1983)
22 Hylden J L K Nahin R L & Dubner R *Brain Res* **411**, 341-350 (1987)
23 Munson J B Collins W F & Mendell L M in *The Current Status of Peripheral Nerve Regeneration* (Eds Gordon T Stein R B & Smith P A) 259-268 (Alan Liss New York 1988)
24 Lewin G R & McMahon S B *Soc Neurosci Abstr* **15**, 13 9 (1989)

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FMRFamide reverses protein phosphorylation produced by 5-HT and cAMP in *Aplysia* sensory neurons

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NEUROTRANSMITTERS can modulate neuronal activity through a variety of second messengers that act on ion channels and other substrate proteins[1-3]. The most commonly described effector mechanism for second messengers in neurons depends on protein phosphorylation mediated by one of three sets of kinases: the cyclic AMP-dependent protein kinases[4,5], the Ca^{2+}–calmodulin-dependent protein kinases[3,6], and the Ca^{2+}–phospholipid-dependent protein kinases[7]. In addition, some neurotransmitters and second messengers can also inhibit protein phosphorylation by lowering cAMP levels (either by inhibiting adenylyl cyclase[8] or activating phosphodiesterases[9]). This raises the question: can neurotransmitters also modulate neuronal activity by decreasing protein phosphorylation that is independent of cAMP? Various biochemical experiments show that a decrease in protein phosphorylation can arise through activation of a phosphatase[10,11] or inhibition of kinases[12,13]. In none of these cases, however, is the physiological role for the decrease in protein phosphorylation known. Here we report that in *Aplysia* sensory neurons, the presynaptic inhibitory transmitter FMRFamide decreases the resting levels of protein phosphorylation without altering the level of cAMP. Furthermore, FMRFamide overrides the cAMP-mediated enhancement of transmitter release produced by 5-hydroxytryptamine (5-HT), and concomitantly reverses the cAMP-dependent increase in protein phosphorylation produced by 5-HT. These findings indicate that a receptor-mediated decrease in protein phosphorylation may play an important part in the modulation of neurotransmitter release.

5-HT and FMRFamide produce opposing modulatory changes in the electrical excitability of the sensory neuron and in transmitter release from the sensory neuron presynaptic terminals[14-16]. By acting through cAMP-dependent phosphorylation, 5-HT produces prolonged all-or-none closure of the S-K^+ channels, which results in a slow depolarization and an increase in the duration of the action potential, contributing to presynaptic facilitation[14]. By contrast, the neuropeptide FMRFamide, acting through the lipoxygenase metabolites of arachidonic acid[17], produces an increase in the probability that an S-channel is open, which results in a slow hyperpolarization and a decrease in the action potential duration, which contributes to presynaptic inhibition[15].

Moreover, the actions of 5-HT and FMRFamide are not symmetrical. Simultaneous application of the two transmitters does not result in simple algebraic summation of excitatory and inhibitory actions, rather, the inhibitory action of FMRFamide antagonizes or overrides the excitatory action of 5-HT. This override occurs at the level of single S-channel activity, where FMRFamide re-opens S-channels closed by 5-HT or cAMP[15]. Here we show that this override is also evident in the action of FMRFamide and 5-HT on transmitter release (Fig. 1*a*). The synaptic potential produced in a motorneuron in response to firing the sensory neuron was decreased by FMRFamide (presynaptic inhibition) to 11% of the control synaptic potential

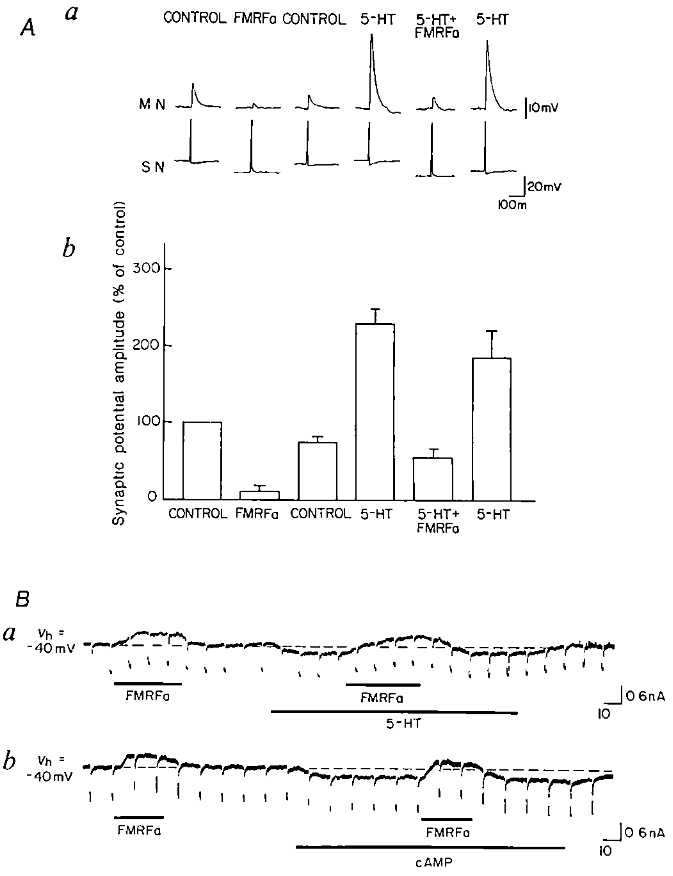

FIG. 1 FMRFamide (FMRFa) antagonizes the actions of 5-HT and cAMP. *A*, FMRFamide produces presynaptic inhibition and antagonizes presynaptic facilitation with 5-HT. *Aa*, Intracellular recordings of membrane potential from motor neurons (top) and sensory neurons (bottom) in response to brief depolarizing current stimuli to sensory neurons. Action potentials in sensory neurons elicit a fast excitatory postsynaptic potential (e.p.s.p.) in motor neurons. *Ab*, Average data from five experiments. Errors bars show s.e.m. Application of 10 µm FMRFamide alone inhibits the synaptic potential recorded in a follower neuron to 11% of that of the initial control. The same dose (10 µm) of 5-HT produces facilitation of the synaptic potential to 229% of that of the control. When FMRFamide is applied in the presence of 5-HT, the synaptic potential is reduced to a level slightly below that of the control (55%). *Ba*, Antagonism by FMRFamide of membrane current response to 5-HT. First application of FMRFamide (10 µm) produces a 450 pA increase in outward S-channel current (V_m = −40 mV). Application of 5-HT then produces a 270 pA decrease in outward S-channel current. Subsequent application of FMRFamide in the presence of 5-HT produces a 660 pA increase in outward current, so that net membrane current approaches peak outward level in FMRFamide alone. *Bb*, Antagonism of current response to cAMP by FMRFamide. V_h, Holding voltage. First application of FMRFamide produces a 420 pA increase in outward current. Next, application of 200 µM chlorophenyl-thio-cAMP causes a 300 pA decrease in outward current. Finally, application of FMRFamide in the presence of cAMP causes a 570 pA increase in outward current. On average, FMRFamide alone causes an increase in outward current of 0.28 nA ± 0.06 (±s.d., n=6, V_m = −40 mV), whereas cAMP alone causes a mean decrease in outward current of 0.2 ± 0.04 nA (±s.d., n=6, V_m = −40 mV). In the maintained presence of cAMP, FMRFamide induces a larger increase in outward current of 0.4 ± 0.007 nA (±s.d., n=6, V_m = −40 mV).

METHODS. *A*, Sensory neurons were grown in cell culture, with their follower motor neurons. Sensory neurons and motor neurons were impaled with a conventional intracellular microelectrode to record membrane potential under current clamp. Action potentials were elicited in the sensory neurons once every 40 s using a 5 ms depolarizing current pulse, and the fast e.p.s.p. were recorded in the motor neuron. *B*, Membrane current was measured from sensory neurons in intact abdominal ganglia by using a single microelectrode switched voltage-clamp (Axoclamp 2A, Axon Instruments). Electrode resistance was 10 MΩ and switching frequency was 6–8 kHz. Current records were filtered at 300 Hz. Hyperpolarizing command voltage-clamp steps of 10 mV were applied at 0.1 Hz.

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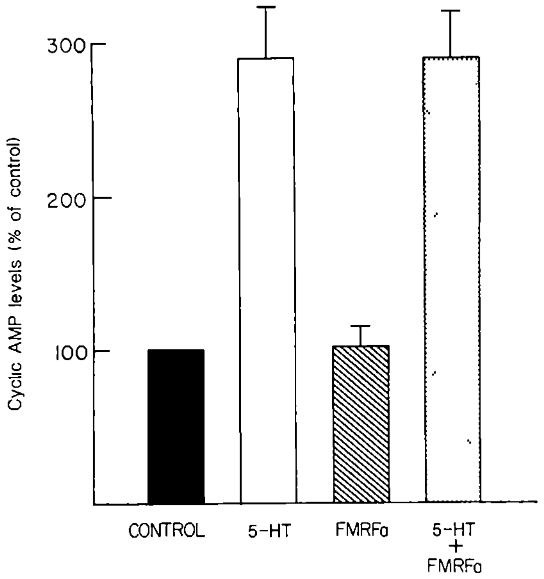

FIG. 2 Cyclic AMP levels in pleural sensory clusters after treatment with 5-HT or FMRFamide (FMRFa). Pleural sensory clusters were stimulated with 40 μM 5-HT for 2 min in the presence of 100 μM IBMX (1 min pre-incubation), or 10 μM FMRFamide with 100 μM IBMX for 1 min. Where 5-HT and FMRFamide were added together, 5-HT was added first for 1 min, then FMRFamide was added and the incubation continued for 1 min. Paired contralateral sensory clusters were used as controls and received no addition. Results shown are means ± s.e.m. for three separate experiments. Addition of IBMX alone cause no significant effect on cAMP levels when assayed at 3 min (control, 100%, IBMX, 79% ± 8%, $n=3$). Previous studies have demonstrated that certain agonists decrease cAMP levels independent of adenylyl cyclase inhibition, at the level of phosphodiesterase activation[9]. The necessity of performing our cAMP assays on cells treated with phosphodiesterase inhibitor precluded us from this directly as a possible mechanism contributing to the inhibitory override.

METHODS. *Aplysia californica* (100–220 g) were obtained from the Marine Biological Laboratories mariculture facility at Woods Hole, Massachusetts. Animals were anaesthetized by injection of approximately one-half of their body weight of isotonic $MgCl_2$. Ring ganglia (cerebral, pleural and pedal ganglia) were removed and pleural sensory neuron clusters, with underlying neuropil, were dissected free (see ref. 19). These bilaterally symmetrical clusters, of ~200 sensory neurons each, were kept as pairs from one animal. Each pair was used for a single experimental determination (assayed in triplicate), with one cluster serving as control and one as treated. This allowed us to perform intra-animal normalization and served to elimiate the inter-animal variability found for basal cAMP levels in *Aplysia* (see also ref. 19). The amount of protein in the left versus the right sensory cluster from one animal (~10 μg) was found to be highly consistent. Thus, comparing left versus right is equivalent to normalizing each sample to protein. Cyclic AMP was assayed using a radioimmunoassay kit (Amersham). Incubations were terminated by the addition of an ice-cold trichloroacetic acid solution to a final concentration of 10%. After 10 min on ice, samples were centrifuged, supernatants collected and ether-extracted, and cyclic AMP assayed. Results were calculated as the ratio of fmol cAMP for experimentals to fmol cAMP for controls. Control samples typically contained 50–75 fmol cAMP per sensory cell cluster.

(s.e.m. = 7.2, $n=5$) and was increased by 5-HT (presynaptic facilitation) to 229% of the control (s.e.m. = 20, $n=5$). When FMRFamide was applied in the presence of 5-HT, the facilitation of the synaptic potential was then overriden and the amplitude of the synaptic potential reduced to 55% of that of the control (s.e.m. = 12, $n=5$).

As expected from the single channel results, the override is also evident in the total membrane current (Fig. 1*b*). Under voltage clamp, application of FMRFamide (10 μM) alone produced an average increase in outward current of 0.23 ± 0.11 nA (±s.d., $n=4$, $V_m = -40$ mV). A mean decrease in outward current of 0.23 ± 0.12 nA ($n=4$) was caused by 5-HT (10 μM) alone. When FMRFamide was applied in the presence of 5-HT, however, the mean FMRFamide-induced current of 0.38 ± 0.10 ($n=4$) was then greater than the FMRFamide-induced current in the absence of 5-HT. FMRFamide therefore not only reverses the decrease in outward current produced by 5-HT, but increases the net current to a level similar to the peak outward current reached with FMRFamide alone. FMRFamide exerts a similar override in the decrease in outward current produced by a membrane-permeable analogue of cAMP (Fig. 1*b*). Thus, at all three levels—transmitter release, membrane current and single channel activity—FMRFamide overrides the action of 5-HT. These results demonstrate that FMRFamide has at least two qualitatively distinct effects in sensory neurons, an inhibitory action when acting on its own, and an overriding action that opposes the action of 5-HT.

How, therefore, does FMRFamide exert its overriding action?

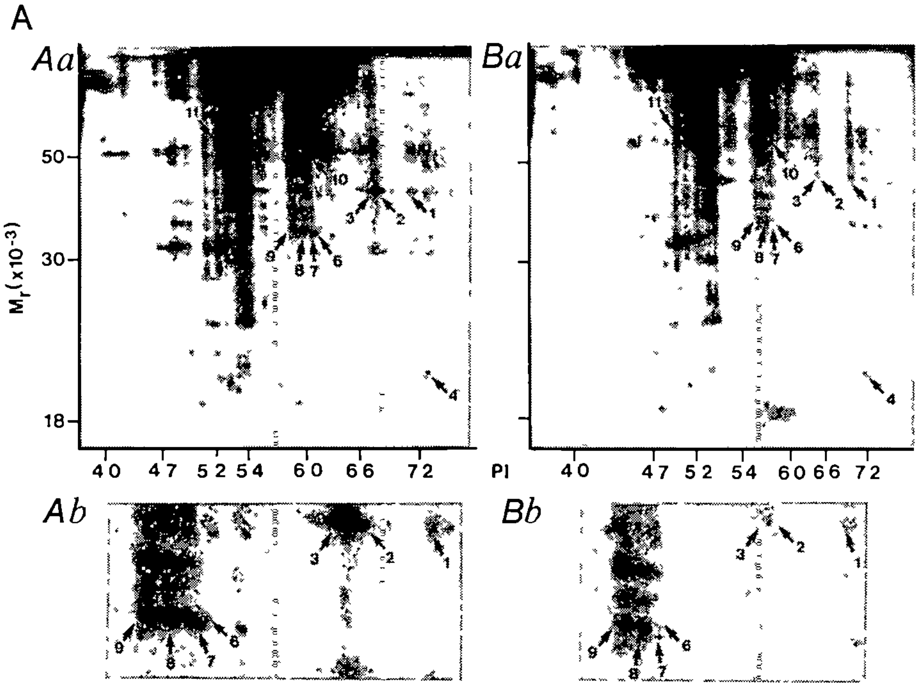

FIG. 3 *A* and *B*, Autoradiographs from contol *Aa* and FMRFamide (*Ba*) treated cells. Autoradiographs are of 2-D gels run on ^{32}P-labelled sensory clusters. Labelling of sensory clusters, 2-D electrophoresis and autoradiography was performed as previously described[20]. *Ab* and *Bb*, Expansion of the central regions of *Aa* and *Bb*. FMRFamide (10 μM) was present for 1 min. In additional experiments (data not shown), no losses of protein spots in response to FMRFamide (10 μM for 1 min) were observed in sensory neurons labelled for 24 h with [^{35}S]methionine (50 μCi per cluster), indicating that the loss of ^{32}P label was not due to a decrease in substrate protein mass.

Specifically, at which stage of the cAMP cascade does FMRFamide exert its antagonistic effect? Three sites of interaction seem possible. First, FMRFamide could override the cAMP cascade by inhibiting the adenylyl cyclase. Second, FMRFamide could stimulate phosphodiesterase activity thereby leading to an increase in the hydrolysis of cAMP (ref. 9). Third, FMRFamide, possibly acting through the metabolites of the lipoxygenase pathway[17], could act at a later stage in the cAMP cascade to reverse phosphorylation of the substrate proteins either by inhibiting cAMP-dependent protein kinase or by activating a phosphoprotein phosphatase. To test these ideas, we studied the interaction of the two cascades at the biochemical level by examining cAMP production and protein phosphorylation.

Although adenylyl cyclase inhibition cannot *per se* explain the antagonism by FMRFamide of the effects of the analogue of cAMP (ref. 15 and Fig. 1*b*), inhibition of the cAMP cascade could still be a contributory mechanism in the antagonism by FMRFamide of the action of 5-HT. We therefore tested the ability of FMRFamide to inhibit adenylyl cyclase by using a radioimmunoassay for cAMP levels in the presence of the phosphodiesterase inhibitor 3-isobutyl-1-methyl-xanthine (IBMX) in *Aplysia* pleural sensory neuron clusters (Fig. 2). 5-HT increased the level of cAMP in *Aplysia* sensory neurons, whereas FMRFamide had no effect, thus confirming previous findings (refs 18 and 19, and T. W. Abrams *et al.*, personal communication). Moreover, FMRFamide did not attenuate the increase in cAMP produced by 5-HT (in agreement with K. A. Ocorr, M. Tabata and J. H. Byrne, personal communication).

These results indicate that the interaction between 5-HT and FMRFamide must occur after the rise in cAMP concentration, perhaps at the level of the phosphorylation and dephosphorylation of substrate proteins. We have previously characterized the effects of 5-HT and cAMP on substrate phosphorylation in the sensory neurons by using an intact-cell protein phosphorylation assay based on quantitative two-dimensional gel autoradiography[20]. We have now used this assay to investigate the effects of FMRFamide on protein phosphorylation, and to search for possible sites of interaction between FMRFamide and the cAMP cascade.

We earlier found that, out of a total of 300–400 phosphoproteins in the sensory neurons resolved by two-dimensional gel electrophoresis[20], 5-HT and cAMP elicit an increase in phosphorylation of 17 proteins. By contrast, we found that FMRFamide elicits a decrease in protein phosphorylation (Figs 3 and 4). Thus, FMRFamide acting alone causes a decrease in the phosphorylation of a subset of 10 of the 17 proteins whose level of phosphorylation is increased by 5-HT or cAMP. No other phosphoproteins resolved on the gel were affected reproducibly. It is unlikely that the decrease in phosphorylation was caused by proteolysis, because autoradiographs from sensory neuron clusters labelled for 24 h with [^{35}S]methionine showed no significant loss of protein spots in response to FMRFamide.

Could a decrease in phosphorylation with FMRFamide contribute to the override of 5-HT's action? To address this question we examined the pattern of protein phosphorylation when FMRFamide was applied in the presence of 5-HT. We found that when FMRFamide is given in the presence of 5-HT it reverses the phosphorylation of all 17 proteins whose phosphorylation is increased in response to 5-HT (Fig. 4). Moreover, FMRFamide also decreased phosphorylation of proteins phosphorylated in response to application of a cAMP analogue. As the experiments with the cAMP analogue were done in the presence of the phosphodiesterase inhibitor IBMX, the decrease in phosphorylation cannot be explained by activation of a phosphodiesterase.

Recent evidence indicates that a single neurotransmitter can exert multiple physiological effects through separate biochemical pathways. Our previous experiments characterized two distinct actions of FMRFamide on S-channel function. Acting on its own, FMRFamide leads to an increase in S-channel open probability (P_o), with no change in the number of active S-channels. When applied in the presence of cAMP or 5-HT, however, FMRFamide also reopens S-channels closed by these agents, leading to an increase in the number of open S-channels in addition to the increase P_o. Our present results indicate that these two actions may be mediated by distinct biochemical mechanisms. The increase in P_o with FMRFamide has been shown to be a direct action of 12-lipoxygenase metabolites of arachidonic acid on the S-channel, independent of protein phosphorylation or dephosphorylation (refs 21, 28). Here we show that FMRFamide also decreases the level of resting protein phosphorylation and reverses the increase in phosphorylation in response to 5-HT and cAMP, thereby providing a probable mechanism for the override of the physiological action of 5-HT and cAMP.

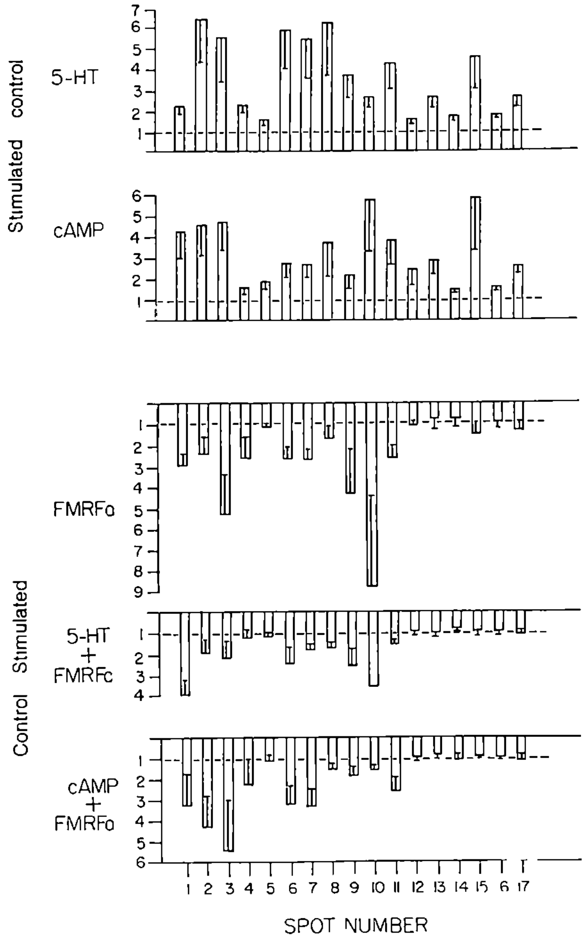

FIG. 4 Effects of various treatments on the phosphorylation of 17 selected proteins from *Aplysia* sensory neurons. Results shown are the means (±s.e.m.) of the ratios of stimulated:control for 5-HT (*n*=6) and cAMP analogue (*n*=7), and control:stimulated for FMRFamide [FMRFa, *n*=7), FMRFa with 5-HT (*n*=4), and FMRFamide with cAMP analogue (*n*=5). Of ~300–400 phosphoprotein spots per gel, only these 17 were found to reproducibly change because of the indicated treatments. Quantitative information for the ^{32}P content of control and stimulated samples was determined by densitometric scanning, and spots were matched and ratios were calculated using the PDQuest computer analysis system[20]. Treatments were 40 μM 5-HT for 2 min, or 100 μm 6-(4-chlorophenylthio)-cyclic AMP with 100 μM phosphodiesterase inhibitor (either IBMX or RO20-1724) for 10 min. Effects on protein phosphorylation were assayed at the end of the stimulus period. Where indicated FMRFamide (10 μM alone or in the presence of 5-HT or cAMP analogue with IBMX) was added 1 min before termination of the incubation. The results shown for 5-HT and cAMP analogue have been reported previously[20].

At present, we cannot distinguish whether this decrease in phosphorylation associated with the override in response to FMRFamide is due to kinase inhibition or phosphatase activation Nor do our experiments by themselves prove a causal link between the decrease in phosphorylation and the override Nonetheless, because the effects of 5-HT and cAMP on S-channel activity in the sensory neurons are mediated by cAMP-dependent phosphorylation, the decrease in phosphorylation with FMRFamide provides a plausible biochemical mechanism for the override

Receptor-mediated decreases in protein phosphorylation (by inhibition of the kinase or activation of a phosphatase) may prove to be a widespread mechanism of cell modulation[22] For example, although the most common mechanism for antagonizing the cAMP cascade is through inhibition of adenylyl cyclase, some mechanisms for antagonism are known to operate in the face of elevated cAMP levels (for reviews, see refs 23-25) Thus, in addition to our previous findings that FMRFamide opens S-channels, there is evidence that in smooth muscle cells acetylcholine can override the effects of cAMP on M-current[26], and in pancreatic islet cells that α_2-adrenergic agonists can inhibit the release of insulin induced by cAMP[27] These examples indicate that a receptor-mediated decrease in phosphorylation could serve in other cell systems to overcome the actions of the cAMP-dependent kinase, or even of other second messenger-dependent kinases □

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1 Kaczmarek L K & Levitan I B (eds) *Neuromodulation* (Oxford University Press New York 1987)
2 Nestler E J & Greengard P *Protein Phosphorylation in the Nervous System* (Wiley New York 1984)
3 Siegelbaum S A & Tsien R W *Trends Neurosci* **6**, 307-313 (1983)
4 Levitan I *J Membrane Biol* **87**, 177-190 (1985)
5 Kuo J F & Greengard P *Proc natn Acad Sci USA* **64**, 1349-1355 (1969)
6 Kennedy M B Bennett M K & Erondu N E *Proc natr Acad Scl USA* **80**, 7357-7361 (1983)
7 Nishizuka Y *Science* **233**, 305-312 (1986)
8 Gilman A G *Cell* **36**, 577-579 (1984)
9 Hughes A R, Martin M W & Harden T K *Proc natn Acad Sci USA* **81**, 5680-5684 (1984)
10 Stewart A A Ingebritsen T S Manalan A Klee C B & Cohen P *FEBS Lett.* **137L**, 80-84 (1982)
11 Hemmings H C Greengard P Tung H Y L & Coher P *Nature* **310**, 503-504 (1984)
12 Walsh D A Perkins J P & Krebs E G *J biol Chem* **243**, 3763-3774 (1968)
13 Walsh D A *et al J biol Chem* **246**, 1977-1985 (1971)
14 Siegelbaum S A Camardo J S & Kandel E R *Nature* **299**, 413-417 (1982)
15 Belardetti F Kandel E R & Siegelbaum S A *Nature* **325**, 153-156 (1987)
16 Kandel E R & Schwartz J H *Science* **218**, 433-443 (1982)
17 Piomelli D *et al Nature* **328**, 38-43 (1987)
18 Bernier L Castellucci V F Kandel E R & Schwartz J H *J Neurosci* **2**, 1682-1691 (1983)
19 Occorr K A & Byrne J H *Neurosci Lett* **55**, 113-118 (1985)
20 Sweatt J D & Kandel, E R *Nature* **339**, 51-54 (1989)
21 Belardetti F *et al Neuron* (in the press)
22 Ingebritsen T S & Cohen P *Science* **221**, 331-338 (1985)
23 Limbird L E & Sweatt J D in *The Receptors* ed P M Conn 281-301 (Academic New York 1985)
24 Kato R & Nakaki T 1983 *Trends pharmacol Sci* **4**, 34-36 (1983)
25 Limbird L E *Fedn Proc* **2**, 2686-2695 (1988)
26 Simms S M Singer J J & Walsh J V *Science* **239**, 190-193 (1988)
27 Nakaki T Kakadate T Yamamoto S & Kato R *Life Sci* **32**, 191-195 (1983)
28 Buttner N Siegelbaum S A & Volterra A *Nature* (in the press)

Inability of CD8α' polypeptides to associate with p56^{lck} correlates with impaired function *in vitro* and lack of expression *in vivo*

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T-CELL accessory molecules, particularly CD4 and CD8, seem to be involved in the control of T-cell activation by antigen. Precisely how such molecules operate is not fully understood, but evidence to date suggests a dual role, as receptors binding ligands on stimulator cells[1,2] and by direct or indirect involvement in intracellular signalling events[3-5] In mouse, truncated 'tailless' CD8 molecules occur naturally (CD8α' polypeptides) and although they are expressed on the surface of thymocytes, they are not expressed on the surface of mature T cells[6] In this study, we show that truncated CD8 molecules are impaired in their ability to interact with the protein tyrosine kinase, p56^{lck}, and have decreased ability to restore immune responsiveness *in vitro* Our data support a dual function for CD8 molecules correlated with expression of external domains and cytoplasmic domains, respectively. Both functions appear to be critical for a competent immune system *in vivo*

We have previously shown that control of surface expression of tailless CD8α' polypeptides in mature peripheral T cells is at the level of protein transport[7] That mature cells should be so stringent in excluding CD8α' chain expression on the cell surface indicates that expression of these tailless molecules may have a negative influence We tested this directly by transfecting individual complementary DNAs encoding CD8α or α' polypeptides into a mouse T-cell hybridoma, DC27 10, which has been transfected with genes encoding the T-cell receptor (TCR) α and β chains specific for class I H-2K^b molecules This hybridoma provides a convenient system in which to assay CD8 function as the transfectant is incapable of responding to antigen in the absence of CD8 molecules, although it will respond to the anti-TCR clonotype antibody, Désiré-1 (ref 8) We selected variants of DC27 10 which lack expression of endogenous CD4 molecules before transfecting CD8 constructs Both α and α' polypeptides can be expressed stably on the surface of this hybridoma in the absence of the CD8β (Ly-3) product (Fig 1*a*) The molecules are expressed as disulphide-bonded homodimers (data not shown) Several individual transfectants were analysed for their ability to respond to anti-clonotypic antibody bound to plastic (Fig 1*b*) and antigen, namely K^b-transfected L cells (Fig 1*c*) Expression of the truncated CD8α' chain is sufficient to permit some restoration of the antigen response Cells expressing the full length CD8α polypeptide, however, respond much better This is particularly apparent if you directly compare a pair of CD8α and α' transfectants which express equivalent but lower levels of both transfected CD8 molecules and T-cell receptors Transfectants a2 and b8, for example, express less CD8 (Fig 1*a*) and respond less well to Désiré-1 antibody (Fig 1*b*) The CD8α transfectant, b8, responds well to antigen in contrast to the CD8α' transfectant, a2, which is completely unresponsive Comparison of transfectants expressing more CD8 and with higher responses to Désiré-1, a1 and b8 2 (Fig 1*a*, *b*), shows that although the CD8α' expressing cells can make some antigen response it is much weaker than that obtained from the CD8α expressing cells (Fig 1*c*) Indeed the antigen response of the brighter CD8α' expressing transfectant is not even as good as that of the duller CD8α expressing transfectant (compare a1 with b8) The fact that some restoration of response can occur in transfectants expressing CD8α' chains indicates that the external domain alone does play a role in the antigen response

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TABLE 1 Hybrid CD8/CD4 molecules respond to antigen only if they contain CD8 extracellular domains

Experiment	Transfectant	Accessory molecule ext	tm	cyt	Response to Desire-1	K^b L cells	K^b spleen
1	a2	CD8	CD8	CD8α'	730	7	—
	b8	CD8	CD8	CD8α	1,085	137	—
	L1	CD8	CD8	CD4	193	87	—
2	a1	CD8	CD8	CD8α'	298	62	—
	b2	CD8	CD8	CD8α	130	265	—
	f6	CD8	CD4	CD4	200	189	—
3	b8 2	CD8	CD8	CD8α	488	512	64
	c27 20	CD4	CD4	CD4	132	0	0
	e9	CD4	CD8	CD8α	329	0	0
	k3	CD4	CD4	CD8α	578	0	0
	j1	CD4	CD4	CD8α'	276	0	0

Transfectants expressing hybrid CD4/CD8 molecules as described in Fig 3, were tested for response to anti-clonotypic antibody Desire-1 (10 μg/ml) 1×10^5 K^b expressing L cells per well or 1×10^6 C57B1/6 anti-thy-1 and complement treated spleen cells per well, as described in Fig 1 Results are expressed as units of interleukin-2 (IL-2) extrapolated from titrating the supernatants on CTLL indicator cells (see Fig 1)

of these accessory molecules in T-cell signalling occurs indirectly through association with this protein tyrosine kinase One possible explanation for the decreased efficacy of the tailless CD8α' chains would be an inability to associate with $p56^{lck}$, and thus participate in intracellular phosphorylation events We tested this by immunoprecipitating CD8α and α' transfectants with anti-$p56^{lck}$ or anti-CD8 antibodies and immunoblotting to assess the presence of co-precipitating $p56^{lck}$ Figure 2*a* clearly demonstrates that in contrast to CD8α chains, CD8α' chains are not associated with $p56^{lck}$ The CD8 molecules on the surface of peripheral T cells are expressed normally as αβ heterodimers, the products of two separate genes (Ly-2 and Ly-3 in mouse) The CD8β chain has a 19 amino-acid cytoplasmic tail and could potentially associate with $p56^{lck}$ We found, however, that this was not the case when we examined cells co-transfected with either CD8α plus β or CD8α' plus β complementary DNA These transfectants show cell-surface expression of CD8αβ or α'β heterodimers respectively, but only the transfectants expressing CD8αβ show association with $p56^{lck}$ (Fig 2*b*) The CD8 α'β heterodimers are present intracellularly in mature murine T cells but are not expressed on the cell surface (R Z, unpublished data) The demonstration that $p56^{lck}$ cannot associate with this heterodimer indicates that expression of molecules capable of $p56^{lck}$ association is critical for an optimally func-

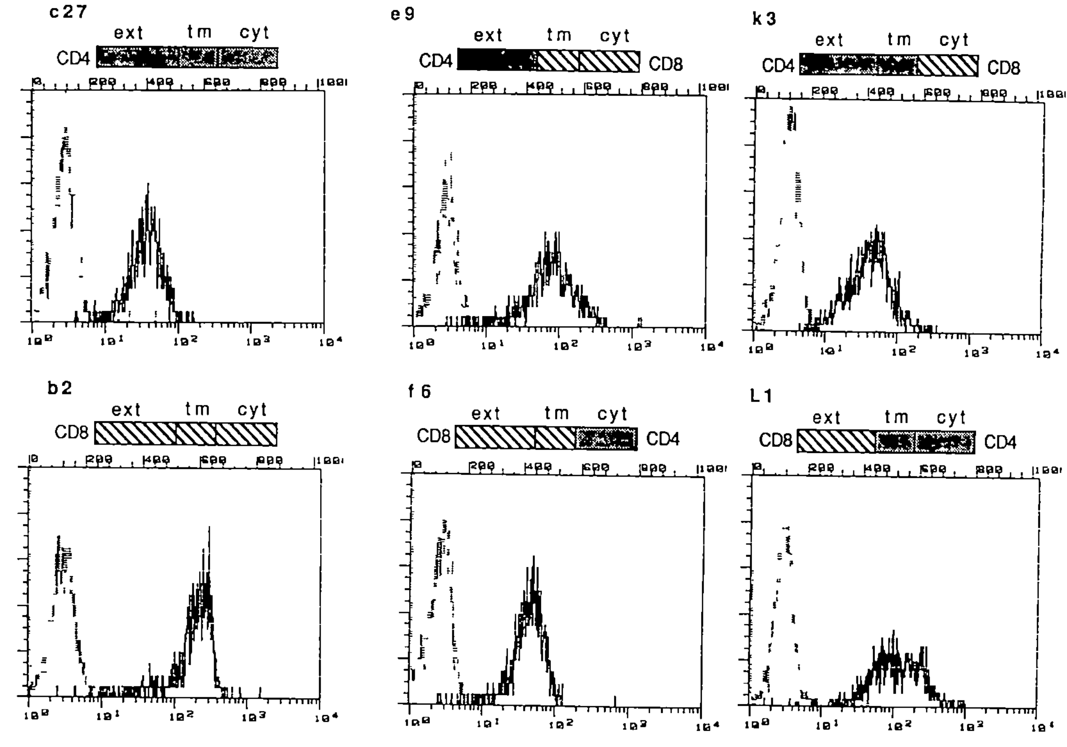

FIG 3 Expression of transfected CD4/CD8 hybrid molecules DC27 10 hybridoma cells were transfected with hybrid cDNA molecules encoding assorted CD4 (filled boxes) and CD8 (dashed boxes) extracellular (ext) transmembrane (tm) and cytoplasmic (cyt) domains, as indicated over each FACS profile Clones expressing CD4 extracellularly, c27, e9 and k3, were stained with FITC-labelled goat anti-rat antibodies alone (dashed profiles) and anti-CD4 (GK1 5) plus FITC-labelled goat anti-rat antibodies (solid profiles) Clones expressing CD8 extracellularly, b2, f6, and L1, were stained with FITC-labelled goat anti-rat antibodies alone (dashed profiles) and anti-CD8 (YTS169 4) plus FITC-labelled goat anti-rat antibodies (solid profiles) Fluorescence intensity, horizontal scale

METHODS Hybrid cDNA molecules were constructed by introducing complementary restriction enzyme sites (*Eco* RV at the extracellular/transmembrane junction, or a *Bst* XII site at the transmembrane/cytoplasmic junction) into CD8 and CD4 cDNAs using site-specific mutagenesis, and then swapping the domains between them Oligonucleotides were designed to minimize amino-acid changes and to maintain the correct reading frame when introducing the restriction enzyme site, although in some cases conservative changes did result No differences were observed in the functions of native and mutated but 'unshuffled' CD4 and CD8 molecules The junctional amino-acid sequences for external/transmembrane domain shuffles were as follows native and mutated CD8-FACD/IYIW , native CD4- VNQT/VFLA , mutated CD4 (transfectant c27)- VNQTD/ILA , ext CD4/tm + cyt CD8 (transfectant e9)- NQTD/IYIW , ext CD8/tm + cyt CD4 (transfectant f6)- FACD/ILA Junctional amino-acid sequences for transmembrane/cytoplasmic domain shuffles were native CD8-LICY/HRSR , native CD4- LCCV/RCRH , mutated CD8 (transfectant b2)- LICY/ARSR , ext + tm CD4/cyt CD8 (transfectant k3)-LCCA/RSRL , ext + tm CD8/cyt CD4 (transfectant L1)- LICYA/RCRH Hybrid cDNAs were subcloned into the β-actin expression vector and transfected as described in Fig 1 tional immune system *in vivo* Given that the α chain alone is able to restore antigen responsiveness, it is not clear why there is a preference for expression CD8$\alpha\beta$ heterodimers rather than CD8$\alpha\alpha$ homodimers on mature T cells It is possible that the CD8β chain has a separate role during the development of $\alpha\beta$ TCR bearing cells in the thymus This would correlate with the observation that intra-epithelial lymphocytes, which are mainly $\gamma\delta$TCR bearing cells[13], predominantly express CD8α homodimers on their surface[14]

The expression of CD4 or CD8 accessory molecules by T cells correlates strongly with whether T cells are restricted by class II or class I MHC molecules[15] Studies in transgenic mice have shown that T cells expressing class I restricted TCR α and β transgenes favour the co-expression of CD8 molecules[16,17] Although this bias may be generated by co-selection of receptor and accessory molecules binding the same class I MHC structure, it is also possible that selection occurs based on preferential interactions between particular TCR and accessory molecule transmembrane or intra-cellular domains We examined this possibility by constructing hybrid cDNA constructs between CD4 and CD8 genes which encode the extra-cellular portion of one together with either the cytoplasmic domain, or the transmembrane plus cytoplasmic domain of the other It was previously shown that expression of CD4 molecules by DC27 10 is insufficient to restore the response to K^b even when stimulator cells express both class II and class I molecules[8] If there is an interaction of the anti-K^b TCR with CD8 in the intra-cellular domains then the hybrids expressing CD4 externally and CD8 internally should be capable of responding to antigen

Transfectants expressing all combinations of hybrid CD4/CD8 molecules were isolated and stained with appropriate monoclonal antibodies (Fig 3) All of the hybrid constructs were expressed stably on the cell surface Immunoprecipitation of these molecules (data not shown) revealed that all constructs expressing CD4 externally were monomers, in contrast to constructs expressing CD8α externally, which were expressed as disulphide bonded homodimers This indicates that interactions between CD8 external domains dictate that this molecule is expressed normally as a dimer Transfectants which gave similar responses to anti-clonotypic antibodies were assayed for their ability to respond to antigen (Table 1) As K^b transfected L cells do not express class II MHC molecules, hybrid constructs expressing CD4 external domains were assayed on H-2K^b spleen cells Despite the presence of the CD4 ligand on the stimulator cells, expression of these hybrid molecules was insufficient to restore antigen response By contrast, transfectants expressing hybrid molecules containing CD8α external domains, whether in combination with CD4 cytoplasmic domains or CD4 transmembrane and cytoplasmic domains, functioned extremely efficiently The latter constructs were at least as effective as native CD8α homodimers in conferring responsiveness to antigen Thus, there does not appear to be preferential association of transmembrane or intracytoplasmic domains of CD8 with a class I specific cell receptor

These data demonstrate an incremental restoration of full antigen responsiveness conferred by transfected CD8 molecules The first step is the progress from complete absence to limited responsiveness conferred by high level expression of CD8 extracellular domains in combination with sufficient expression of TCRs This property can be ascribed to increased avidity stabilizing the interaction between the T cell and the antigen presenting cell It is interesting to note that in contrast to the experiments reported by Gay *et al*[18], our experimental system has an absolute requirement for both the TCR and accessory molecules to recognize class I MHC The Désiré-1 TCR has low affinity for antigen[19] and may only be stabilized by CD8 external domains binding to the same class I molecules as the TCR This requirement may not be so obvious for higher affinity TCRs

The second increment by which CD8 influences responsiveness relates to the presence of the cytoplasmic domain and correlates with the ability to associate with p56^{lck} This association has been demonstrated for both CD4 and CD8 molecules and can explain why CD4 intracellular domains are efficient substitutes for CD8 intracellular domains These results are in contrast to hybrid CD8 molecules with class I cytoplasmic domains which are only as efficient as CD8α' chains in restoring responsiveness (B Malissen, personal communication) □

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1 Doyle C & Strominger J L *Nature* **330**, 256 (1987)
2 Norment A M Salter R D Parham P Engelhard V H & Littman D R *Nature* **336**, 79-81 (1988)
3 Emmrich F Strittmatter U & Eichmann K *Proc natn Acad Sci USA* **84**, 8298-8302 (1986)
4 Ledbetter J A *et al Eur J Immun* **18**, 525-532 (1988)
5 Jonsson J I Boyce N W & Eichmann K *Eur J Immun* **19**, 253-260 (1989)
6 Walker I D *et al Immunol Rev* **82**, 47 77 (1984)
7 Zamoyska R & Parnes J R *EMBO J* **7**, 2359-2367 (1988)
8 Gabert J *et al Cell* **50**, 545-554 (1987)
9 Rosenstein Y Ratnofsky S Burakoff S J & Herrmann S J *J exp Med* **169**, 149-160 (1989)
10 Rudd C E Trevellyan J M Dasgupta J D Wong L L & Schlossman S F *Proc natn Acad Sci USA* **85**, 5190-5196 (1988)
11 Veillette A Bookman M A Horak E & Bolan J B *Cell* **55**, 301-308 (1988)
12 Veillette A Bookman M A Horak E Samelson L E & Bolan J B *Nature* **338**, 257-259 (1989)
13 Goodman T & LeFrancois L *Nature* **33**, 855-858 (1988)
14 Parrott D M V *et al Ann NY Acad Sci* **409**, 307-320 (1983)
15 Swain S L *Immunol Rev* **74**, 129-142 (1983)
16 Kisielow P Teh H S Bluthmann H & von Boehmer H *Nature* **335**, 730-733 (1988)
17 Sha W C *et al Nature* **336**, 73-76 (1988)
18 Gay D *et al Nature* **328**, 626-629 (1987)
19 Hua C Boyer C Guimenzanes A Alpert F & Schmitt-Verhulst A M *J Immun* **136**, 1927-1936 (1986)
20 Oi V T Morrison S L Herzenberg L A & Berg P *Proc natn Acad USA* **80**, 825-829 (1983)
21 Gunning P Leavitt J Muscat G Ng S Y & Kedes L *Proc natn Acad Sci USA* **84**, 4831-4835 (1987)
22 Zamoyska R Vollmer A C Sizer K C Liaw C W and Parnes J R P *Cell* **43**, 153-163 (1985)
23 Ledbetter J A & Herzenberg L A *Immunol Rev* **47**, 63-90 (1979)

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Genetic imprinting suggested by maternal heterodisomy in non-deletion Prader-Willi syndrome

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PRADER-WILLI syndrome (PWS) is the most common form of dysmorphic genetic obesity associated with mental retardation[1,2] About 60% of cases have a cytological deletion of chromosome 15q11q13 (refs 2, 3) These deletions occur *de novo* exclusively on the paternal chromosome[4,5]. By contrast, Angelman syndrome (AS) is a very different clinical disorder and is also associated with deletions of region 15q11q13 (refs 6–8), indistinguishable from those in PWS[6,8] except that they occur *de novo* on the maternal chromosome[6] The parental origin of the affected chromosomes 15 in these disorders could, therefore, be a contributory factor in determining their clinical phenotypes. We have now used

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cloned DNA markers specific for the 15q11q13 subregion[5,9,10] to determine the parental origin of chromosome 15 in PWS individuals not having cytogenetic deletions, these individuals account for almost all of the remaining 40% of PWS cases Probands in two families displayed maternal uniparental disomy for chromosome 15q11q13 This is the first demonstration that maternal heterodisomy—the presence of two different chromosome 15s derived from the mother—can be associated with a human genetic disease. The absence of a paternal contribution of genes in region 15q11q13, as found in PWS deletion cases[4,5], rather than a mutation in a specific gene(s) in this region may result in expression of the clinical phenotype Thus, we conclude that a gene or genes in region 15q11q13 must be inherited from each parent for normal human development.

Given the unusual parental origins of deletion chromosomes associated with PWS and AS, we attempted to determine the parental origins of chromosomes 15 in non-deletion cases of PWS For each family, we performed restriction fragment length polymorphism (RFLP) analyses with nine RFLPs at seven loci specific for proximal chromosome 15q (refs 5, 11) The first family that we studied has a proband with a phenotype typical of PWS (refs 1, 2), that is, hypotonia and growth delay in infancy, short stature, hyperphagia with consequent obesity, small hands and feet, hypogonadism and mild mental retardation This family also displays inheritance of a balanced robertsonian translocation, t(13, 15) (Fig 1*a*) Association of this translocation with the phenotype of PWS is highly unlikely because unaffected maternal relatives carry the same balanced translocation (Fig 1*a*), and the translocation breakpoint is at the centromere of chromosome 15, some distance from the critical region at 15q11q13 In this family the PWS proband (PWS1) had inherited two maternal alleles but no paternal allele (Fig 1*b*) specific for probe 3-21, which maps to the region absent in PWS patients with deletions[5,9 10] The 8 2-kilobase (kb) *Taq*I fragment detected by probe 3-21 (Fig 1*b*) is shared by the three individuals (PWS1, his mother M1, and half-sister) carrying the t(13, 15) translocation and is, therefore, a marker for this chromosome The 8 9-kb *Taq*I fragment which hybridized to probe 3-21 is present in both PWS1 and his mother and is not present in the other individuals of the family, clearly indicating the maternal derivation of the non-translocated chromosome 15 in PWS1, at least for region 15q11q13 surrounding the probe 3-21 locus We confirmed and extended this result by using CMW-1, a multiallelic DNA marker[11] that maps distal to the region of deletions associated with PWS (unpublished result) The segregation pattern again showed two maternal alleles but no paternal allele for the proband (Fig 1*c*) We excluded the possibility of non-paternity by analysis with a cloned fragment (3'HVR) from the 3' hypervariable region of the α-globin locus on chromosome 16 (ref 12, Fig 1*d*)

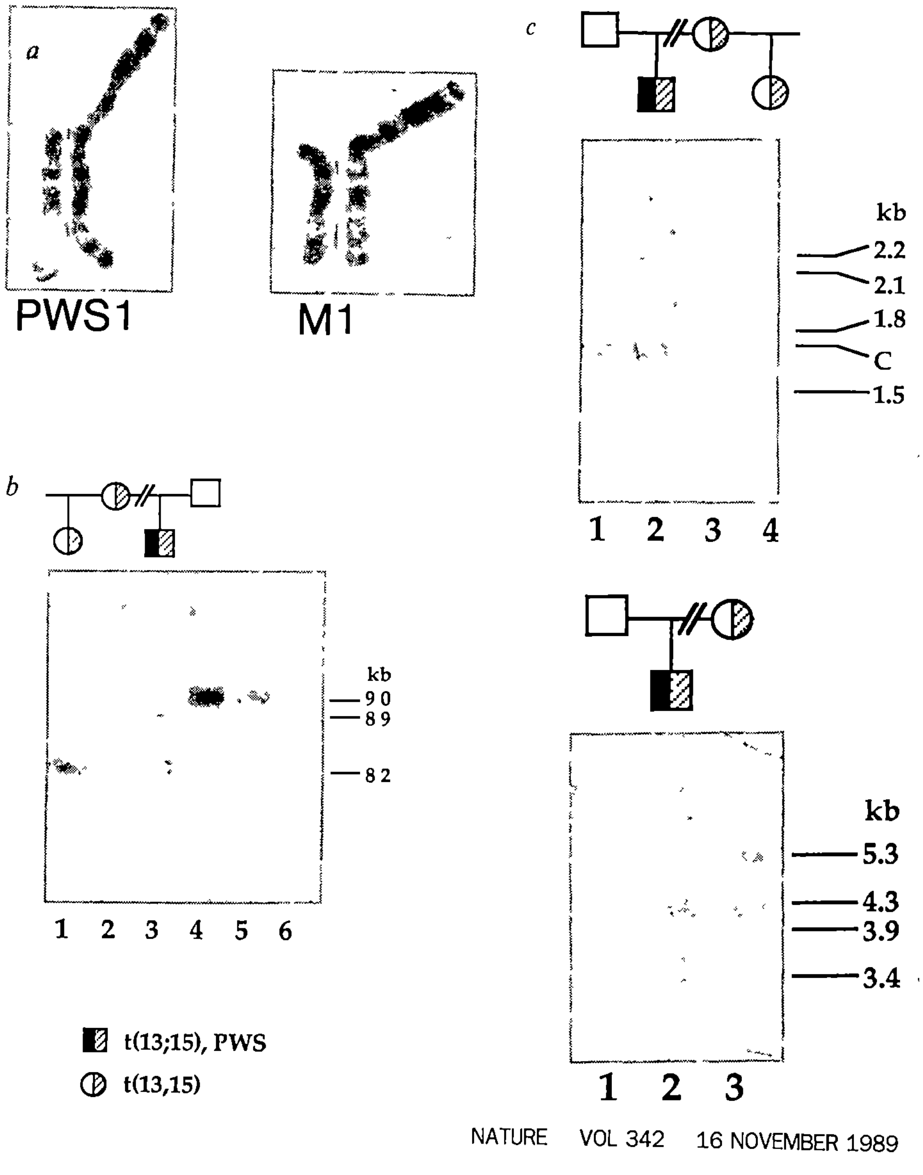
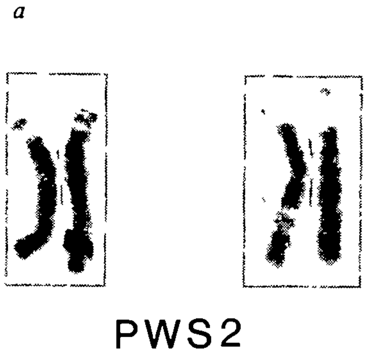
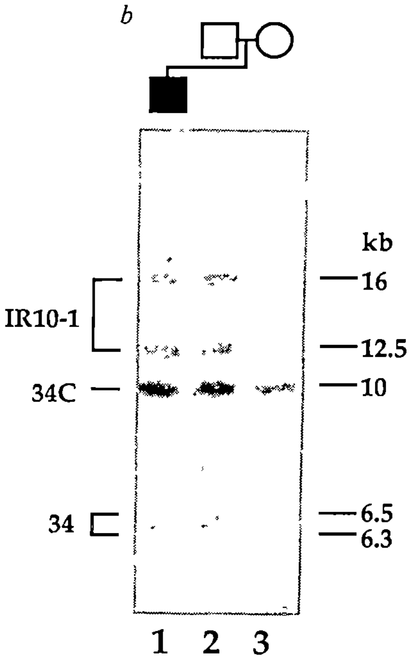


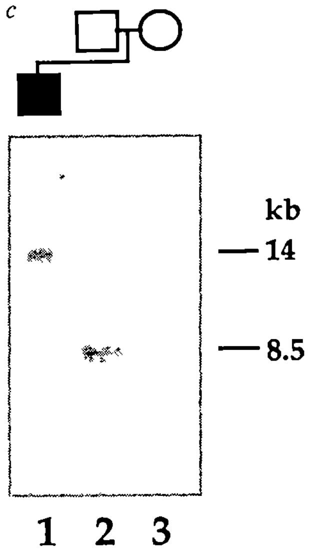
FIG 1 Maternal uniparental disomy in a PWS patient with a balanced Robertsonian translocation *a*, Normal chromosome 15 and translocated homologue from patient PWS1 (left) and his mother M1 (right) Other unaffected maternal relatives, including the half-sister of PWS1 carry the same balanced chromosome 13q15q robertsonian translocation *b*, Maternal origin of the two chromosomes 15 in patient PWS1 An 8 2-kb *Taq*I allele of probe 3-21 (D15S10) is shared by PWS1 (lane 3), his mother M1 (lane 2) and half-sister (lane 1), whereas an 8 9-kb band is shared by PWS1 and M1 PWS1 does not inherit a 9 0-kb paternal allele (F1, lane 4) Mixing experiments (lane 5, PWS1 and F1, lane 6, M1 and F1) show the identity of the three alleles in this new RFLP system *c*, Presence of two maternal alleles only for CMW-1 (D15S24) in PWS1 PWS1 (lane 2) shares two *Taq*I restriction fragments, of 2 1 and 2 2 kb, with M1 (lane 3) but has neither of the 1 5- or 1 8-kb paternal alleles (F1, lane 1) The half-sister (lane 4) displays the maternal 2.1-kb and a 2 15-kb allele The band at 1 7 kb is constant, C *d*, Exclusion of non-paternity for PWS1 *Pvu*II-digested DNA was hybridized to the α-globin 3'HVR marker (D16S85), one of the most highly polymorphic markers in the human genome[12] PWS1 (lane 2) shows mendelian inheritance of one paternal 3 9-kb allele (lane 1) and one maternal 4 3-kb allele (lane 3) The half-sister inherits her mother's 5 3-kb allele (result not shown)

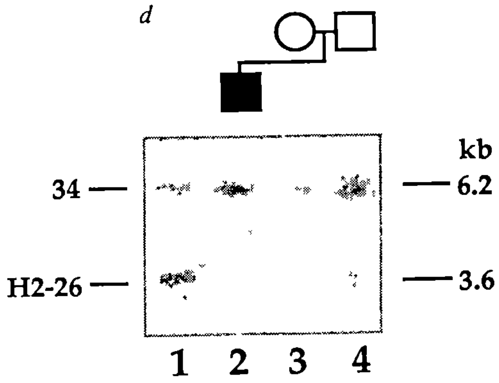
METHODS DNA was isolated from blood and/or cell lines from each individual as described previously[5] Hybridizations were carried out as previously described[5] High-resolution chromosome analyses from peripheral blood lymphocytes[29] were performed by Giemsa-trypsin banding[30]

The proband (PWS2) of the second family also has classical PWS (refs 13, 14) Cytogenetically, PWS2 has two intact chromosome 15s (Fig 2*a*) We demonstrated the lack of paternal alleles for two loci by haplotype analysis (Fig 2*b*, *c*) With probe 34, the father is homozygous for 6 5 kb alleles, whereas the mother is homozygous for 6 3 kb alleles (Fig 2*b*) For the proband, we found only a 6 3-kb band (Fig 2*b*), consistent with the maternal origin of both cytogenetically normal chromosomes 15 Likewise, only maternal alleles specific for probe IR39d had been inherited (Fig 2*c*) By dosage analyses we demonstrated that PWS2 has two copies of each of these maternally derived alleles (Fig 2*d* and results not shown), indicating that loci detected by probes 34 and IR39d had not been deleted These two loci are flanked by loci detected by probe IR4-3R (results not shown) and probe IR10-1, respectively, (Fig 2*b*) that are heterozygous in the proband To rule out the possibility of a submicroscopic deletion, we analysed the long-range structure of the 15q11q13 region by pulsed-field gel electrophoresis (PFGE) This showed that probe IR4-3R and probe 34 detect the same large unaltered 2,500-kb *Not*I fragment in PWS2 (Fig 3), which provides strong evidence that the absence of a paternal allele specific for probe 34 does not represent a microdeletion Furthermore, we detected a band corresponding to a fragment of the same size (2,500 kb) in the mother, despite differential methylation leading to partial









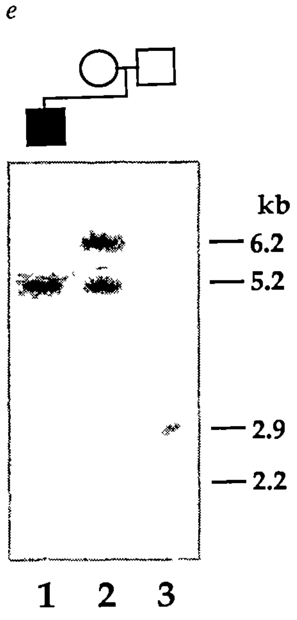


FIG 2 Maternal uniparental disomy in a PWS patient with apparently normal chromosomes 15 *a*, Two pairs of intact chromosomes 15 of PWS2 Previous reports have indicated these chromosomes as being intact[13] or deleted 15q (ref 14), in the deletion report a polymorphic shortening of 15q11 (ref 7) was probably detected, because we show here at a higher level of resolution that the chromosomes are intact *b*, Maternal origin of the two chromosomes 15 in PWS2 DNA was digested with *Sca*I PWS2 (lane 1) inherits a maternal 6 3-kb allele (lane 3) but no paternal 6 5-kb allele (lane 2) for probe 34 (D15S9) Each individual is heterozygous for IR10-1 (D15S12, 16- and 12 5-kb alleles), which is from the same 15q11q13 region as probe 34 (ref 10) The band at 10 kb is constant, C, for probe 34 in all individuals[5] *c*, PWS2 inherits maternal alleles only for probe IR39d (D15S18) DNA was digested with *Sac*I and hybridized with the 15q11q13-specific probe IR39d PWS2 (lane 1) inherits his mother's (lane 3) 14-kb alleles and not his father's (lane 2) 8 5-kb alleles *d*, Two copies of the maternal allele for probe 34 in PWS2 *Hin*dIII-digested DNAs from PWS2 (lane 2), mother M2 (lane 3), father F2 (lane 4) and a PWS patient displaying both a cytological and molecular deletion of 15q11q13 (ref 5, lane 1, HS2) were hybridized simultaneously with probes 34 and H2-26 (D13S28)[31] Probe H2-26 from chromosome 13 serves as a reference for quantitation of copy number[10] Probe 34 shows a reduced hybridization intensity relative to H2-26 only in HS2, which is consistent with a deletion of 15q11q13 in this individual No reduction in probe 34 hybridization intensity is observed in PWS2 or his parents To quantitate the intensity of the hybridization bands, the autoradiogram was scanned with an LKB Ultrascan XL laser densitometer (Pharmacia LKB Biotechnology) The area ratio of the probe 34 to probe H2-26 peaks was calculated for each lane The number of copies of probe 34 per genome was obtained by setting the ratio in lane 3 (M2, mother) to 2 and normalizing the values in the other lanes to this value The number of copies of probe 34 per genome determined by this method were 1 0 in lane 1 (the HS2 deletion), 1 8 in lane 2 (PWS2) and 2 2 in lane 4 (F2, father) *e*, Exclusion of non-paternity for PWS2 One maternal 5 2-kb (lane 2) and one paternal 2 9-kb (lane 3) *Pvu*II allele are inherited by PWS2 (lane 1) for the 3'HVR

METHODS DNA and cytogenetic studies were as for Fig 1 Probe IR39d is a subclone of IR39 (ref 10), which lacks repetitive sequences PWS2 has been described before as patient number 5 (ref 14) and patient number 31 (ref 13)

*Not*I digestion in this region of the genome (Fig 3) and in other normal individuals (results not shown) We again excluded the possibility of non-paternity using the 3′HVR fragment, which detected an entirely different set of alleles in the PWS2 family (Fig 2*e*) from those that it detected in the PWS1 family (Fig 1*d*)

The results presented here indicate that both PWS probands inherited two different, intact chromosome 15q11q13 regions from their mothers Maternal heterodisomy—a newly defined form of uniparental disomy[15,16]—for at least the critical region of chromosome 15, could thus have an aetiological role in PWS The extent of disomy remains to be determined by genetic analysis with other markers The disomy extends beyond the PWS critical region identified from deletion studies because locus CMW-1, which maps distal to the deletion region, is disomic in PWS1 Several predictions can be made about an association between uniparental disomy and the aetiology of PWS First, there should be no chromosome deletion, as is shown in this study by using both cytogenetic and molecular genetic techniques Additional PFGE mapping data for PWS1 and PWS2 (Fig 3 legend) confirmed that a large part (4–5 megabases) of the 15q11q13 region is intact in these two patients Second, there should be no specific gene mutation Although point mutations cannot be ruled out until the gene(s) responsible for PWS are isolated, these seem unlikely because each case displays two independent maternal contributions to region 15q11q13 and the disorder is genetically dominant, which would rule out mechanisms such as the uncovering of a recessive mutation Finally, the frequency of maternal disomy should be high in PWS patients with normal chromosomes Consistent with this prediction, our preliminary data on four additional families strongly indicates that all of the PWS patients show maternal uniparental disomy

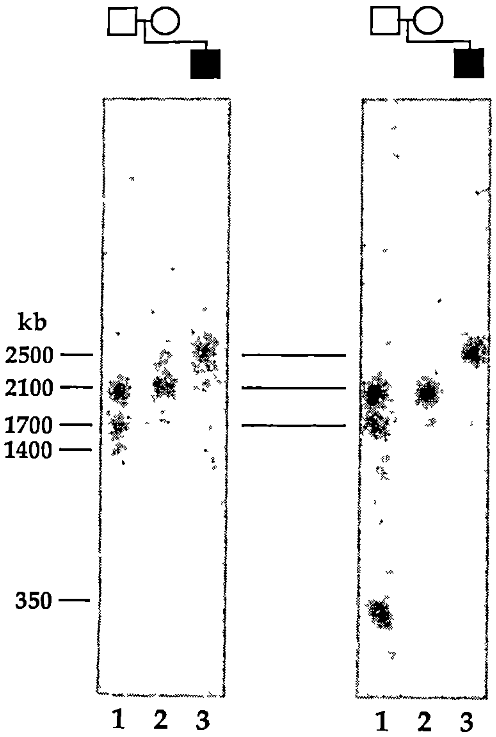


FIG 3 PFGE provides evidence for the lack of a submicroscopic deletion in PWS patients with uniparental disomy *Not*I fragments are shown for probes IR4-3R (D15S11) (left) and 34 (right) in PWS2 (lane 3), M2 (lane 2) and F2 (lane 1) Multiple bands are indicative of partial cleavage at restriction sites when CpG is methylated[32] and a similar pattern of multiple bands has been observed in other normal controls (results not shown) PWS2 shares a normal 2,500-kb band with his mother, although in the latter this results from partial digestion at a *Not*I site in this region of the genome The same 2,500-kb band has been detected in other normal individuals The larger three fragments are common to probes IR4-3R, 189-1 (results not shown) and 34, showing that these loci are closely linked in region 15q11q13 These probes and a 1,300-kb fragment detected with probes 3-21 and IR10-1 (unpublished data) are deleted in all PWS[10] and AS[6] patients with cytological deletions Additional PFGE analysis (results not shown) also indicates that PWS2 displays PFGE restriction fragments of normal size for IR10-1 in a *Not*I digest (1,300 kb) and for IR39d in a *Bss*HII digest (250 kb) PWS1 also seems to be intact for the probe 34 and 3-21 loci because the restriction fragments detected using *Not*I and *Bss*HII are unaltered compared with those of normal individuals

METHODS PFGE analyses were performed as described previously[33] using a modification of techniques for DNA preparation and digestion in agarose blocks[34] Electrophoresis was performed in 0 8% agarose in TBE (89 mM Tris buffer, pH 8 0, 89 mM boric acid, 2 mM EDTA) at 22 °C and was divided into three 48-h intervals during which the forward polarity pulse durations were varied from 75–600 s, 600–2,500 s and 2,500–3,000 s and field intensities were set at +2, +1 7 and +1 3 V cm^{-1}, respectively The ratios of the duration and intensity of the inverse polarity pulse relative to the forward were 0 4 and 0 5, respectively

It seems that the clinical phenotype of the two PWS probands arises from the absence of a paternal contribution to region 15q11q13, rather than from a specific gene mutation This implies functional differences in alleles of a gene or genes from this region of the genome that depend on the sex of the transmitting parent (genetic imprinting[18-21]) Under this scheme, normal human development would require genetic input from both parents, whereas the absence of a paternal contribution to region 15q11q13, whether by paternal deletion[4,5] or maternal uniparental disomy (as demonstrated here), would result in PWS Consistent with this hypothesis is our recent finding of differential transmission of parental alleles in PWS and AS (ref 6) It is conceivable, therefore, that the absence of a maternal contribution to the same 15q11q13 region could result in a different disorder, AS

Experimental evidence for genetic imprinting has been largely restricted to the mouse in which a requirement for a contribution of both the maternal and paternal genome for normal development has been demonstrated[18-22] Transgenic studies have provided preliminary evidence that the chromatin alteration involved in the differential modification and expression of parental alleles is DNA methylation[18-22], a process also associated with transcriptional regulation of gene activity[23] In humans, evidence of a developmental requirement for both parental genomes is provided by the finding that only the paternal genome is present in complete hydatidiform moles[24] Furthermore, differential transmission of parental alleles in several disorders such as Huntington's chorea[18,25], and somatic changes in childhood tumours such as Wilms tumour, osteosarcoma (reviewed in ref 26) and rhabdomyosarcoma[27] could involve genome imprinting Growth failure in two individuals with isodisomy[15] for chromosome 7 (refs 16, 28) could indicate genetic imprinting[16], although the aetiology of cystic fibrosis (CF) in these patients is homozygosity for a mutant CF allele inherited from the mother[16 28]

In conclusion, the association of PWS with maternal uniparental disomy for region 15q11q13 implicates a role for genetic imprinting in the aetiology of the PWS phenotype Similar phenomena could contribute to other clinical syndromes Isolation of the gene(s) responsible for PWS and other disorders in which genetic imprinting plays a part might be possible by identifying a gene that shows a differential modification of its parental alleles Our findings for PWS represent a step towards understanding the developmental basis of this common human genetic disorder ☐

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1 Cassidy S B *Curr Problems Pediatr* **14,** 1–55 (1984)
2 Butler M G *Am J med Genet* (in the press)
3 Ledbetter D H Greenberg F Holm V A & Cassidy S B *Am J med Genet* **28,** 779–790 (1987)
4 Butler M G Meaney F J & Palmer C G *Am J med Genet* **23,** 793–809 (1986)
5 Nicholls R D *et al Am J med Genet.* **33,** 66–77 (1989)
6 Knoll J H M *et al Am J med Genet* **32** 285–290 (1989)

7. Pembrey, M. *et al. J. Med. Genet.* **26,** 73–77 (1989).
8. Donlon, T. A. *Hum. Genet.* **80,** 322–328 (1988).
9. Donlon, T. A., Lalande, M., Wyman, A., Bruns, G. & Latt, S. A. *Proc. natn. Acad. Sci. U.S.A.* **83,** 4408–4412 (1986).
10. Tantravahi, U. *et al. Am. J. med. Genet.* **33,** 78–87 (1989).
11. Rich, D. C., Witkowski, C. M., Summers, K. M., van Tuinen, P. & Ledbetter, D. H. *Nucleic Acids Res.* **16,** 8740 (1988).
12. Higgs, D. R. *et al. Proc. natn. Acad. Sci. U.S.A.* **83,** 5165–5169 (1986).
13. Butler, M. G. & Meaney, F. J. *Am. J. med. Genet.* **26,** 445–455 (1987).
14. Butler, M. G. *Am. J. hum. Genet.* **45,** 140–146 (1989).
15. Engel, E. *Am. J. med. Genet.* **6,** 137–143 (1980).
16. Spence, J. E. *et al. Am. J. hum. Genet.* **42,** 217–226 (1988).
17. Warburton, D. *Am. J. hum. Genet.* **42,** 215–216 (1988).
18. Solter, D. *A. Rev. Genet.* **22,** 127–146 (1988).
19. Sapienza, C. *Annls N.Y. Acad. Sci.* (in the press).
20. Monk, M. *Genes Dev.* **2,** 921–925 (1988).
21. Holliday, R. *Sci. Am.* **260,** 60–73 (1989).
22. Surani, M. A., Reik, W. & Allen, N. D. *Trends Genet.* **4,** 59–62 (1988).
23. Cedar, H. *Cell* **53,** 3–4 (1988).
24. Kajii, T. & Ohama, K. *Nature* **268,** 633–634 (1977).
25. Reik, W. *J. med. Genet.* **25,** 805–808 (1988).
26. Reik, W. & Surani, M. A. *Nature* **338,** 112–113 (1989).
27. Scrable, H. *et al. Proc. natn. Acad. Sci. U.S.A.* (in the press).
28. Voss, R. *et al. Am. J. hum. Genet.* **45,** 373–380 (1989).
29. Yunis, J. J. *Science* **191,** 1268–1270 (1976).
30. Seabright, M. *Lancet* **ii,** 971–972 (1971).
31. Lalande, M. *et al. Cancer Genet. Cytogenet.* **13,** 283–295 (1984).
32. Fischel-Ghodsian, N., Nicholls, R. D. & Higgs, D. R. *Nucleic. Acids Res.* **15,** 6197–6207 (1987).
33. Lalande, M., Noolandi, J., Turmel, C., Rousseau, J. & Slater, G. W. *Proc. natn. Acad. Sci. U.S.A.* **84,** 8011–8015 (1987).
34. Schwartz, D. C. & Cantor, C. R. *Cell* **37,** 67–75 (1984).

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Requirement for integrins during *Drosophila* wing development

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THE position-specific (PS) integrins of *Drosophila*[1,2] are highly homologous to vertebrate integrins[3–5], most of which are cell-surface receptors for extracellular matrix components[6,7]. Integrins are heterodimers, each consisting of noncovalently associated α- and β-subunits. As for the subfamilies of vertebrate integrins, the same β-subunit is found in both *Drosophila* PS integrins, combined with a specific α-subunit to generate either a complete functional PS1 or PS2 integrin[1,5,8]. Both α- and β-subunits are large transmembrane proteins (relative molecular masses >100,000). Either one or both of these two PS integrins are expressed in most fly tissues during development. A particularly intriguing pattern of expression is found in the mature wing imaginal disc, where the PS1 integrin is expressed primarily on the presumptive dorsal wing epithelium, and the PS2 integrin is found almost exclusively on the ventral epithelium[1]. Immediately after pupariation, the central wing pouch evaginates, folding along its centre to appose the epithelia that will secrete the dorsal and ventral surfaces of the adult wing blade[9]. Here we report the results of a genetic analysis indicating that both of the PS integrins are required to maintain the close apposition of the dorsal and ventral wing epithelia during morphogenesis. Also, we conclude that the integrins are not necessary for the maintenance of the cell lineage restriction between the two presumptive wing surfaces in the developing imaginal disc[10–12].

The α-subunit of the PS2 integrin seems to be encoded by the *inflated* (*if*) locus on the X chromosome[13], and null mutations at *if* cause embryonic lethality (M. Wilcox, A. DiAntonio and M. Leptin, manuscript submitted; see also Fig. 2 legend). We examined if^3 mutant larval tissues by using monoclonal antibodies directed against the PS integrins (anti-PS antibodies), and our results suggest that the if^3 allele is a regulatory mutation at the locus. Homozygous if^3 late third-instar larvae (just before pupariation and disc evagination) had apparently normal levels of PS2 integrin on most tissues (for example, muscles, salivary glands), but displayed greatly reduced levels of integrin on the surfaces of some imaginal disc cells (Fig. 1*b*). Specifically, there was relatively little PS2 integrin α-subunit in the ventral region of the wing pouch; these cells normally express the protein at very high levels. PS2 integrin expression was not so severely reduced in other regions of the disc, such as the peripodial membrane. The expression of the PS1 integrin in the wing disc seemed to be unaffected by if^3 (not shown).

Although all of the mutant wing discs examined were clearly affected, there was variability in the extent of PS2 integrin reduction (for example, Fig. 1*b*, *c*). In the wing pouch, mutant discs typically stained at higher levels anteriorly, and often displayed significant levels of staining with anti-PS2 antibody dorsally (Fig. 1*c*). Similar antero-posterior asymmetry and dorsal staining are characteristic of wild-type PS2 integrin expression in mid-third instar discs[14], about 24 h before pupariation. It is interesting that the pattern of PS2 integrin expression in if^3 discs seemed relatively normal at the mid-third larval instar (not shown), and it is possible that this allele simply does not allow the cells of the pouch to progress to the more mature pattern of expression.

Adult flies bearing the if^3 allele display wing blisters, in which the dorsal and ventral surfaces of the wing blade are separated[13]. We found that the penetrance and expressivity of the if^3 blisters were variable (Fig. 2); this is not surprising in light of the variability of the immunofluorescence results. Nonetheless, these data indicate that the PS2 integrin is important for maintaining the close apposition of the dorsal and ventral epithelia during morphogenesis of the adult wing.

That this is the case was confirmed by clonal analysis studies[15] of flies with mutations in the *myospheroid* gene (*mys*). The *mys* locus encodes the common PS integrin β-subunit[4,5], so null mutations at this locus would be expected to eliminate both PS1 and PS2 integrins[5]. Flies homozygous for null mutations of *mys* die as embryos (see refs 16 and 17 for descriptions of the *mys* lethal phenotype), so to examine the role of integrins in wing morphogenesis, we made clones of cells homozygous for a null mutation of *mys*, along with the flanking markers *yellow* (*y*) and

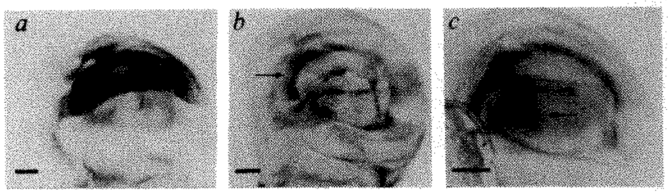

FIG. 1 Anti-PS2-antibody immunofluorescence of the basal surfaces of late third-instar wing imaginal discs. At this stage, the disc consists primarily of a single layered but highly folded columnar epithelium of ~50,000 cells[22]. All micrographs are centred on the 'pouch' region, which evaginates into a flat sac before secreting the cuticle of the adult wing blade[9]. Images are reverse-contrast, so staining is dark. *a*, Wild type. PS2 integrin is found at high concentration throughout the ventral (upper) region of the wing pouch. The sharp horizontal boundary of staining across the pouch marks the line along which the epithelium will fold during evagination (that is, the presumptive wing margin). *b*, Mutant for if^3. PS2 expression is greatly reduced in the pouch. Relatively high levels of staining remain in more peripheral areas, especially along the anterior edge of the pouch (arrow). *c*, Mutant for if^3. Higher magnification of another disc with a less extreme phenotype, showing residual PS2 in the pouch. Typically, expression is greater in the anterior (left) half of the pouch, and is often seen dorsally (arrow) as well as ventrally. Scale bar, 50 μm.

METHODS. Mutants for if^3 were grown as a homozygous stock at 25 °C. Immunofluorescence of wing discs was performed with an anti-PS2 (CF.2C7) monoclonal antibody and fluorescein-conjugated goat anti-mouse antibody (Antibodies Inc.) as previously described[1]. Images were detected with an ISIT video camera, and stored on video tape.

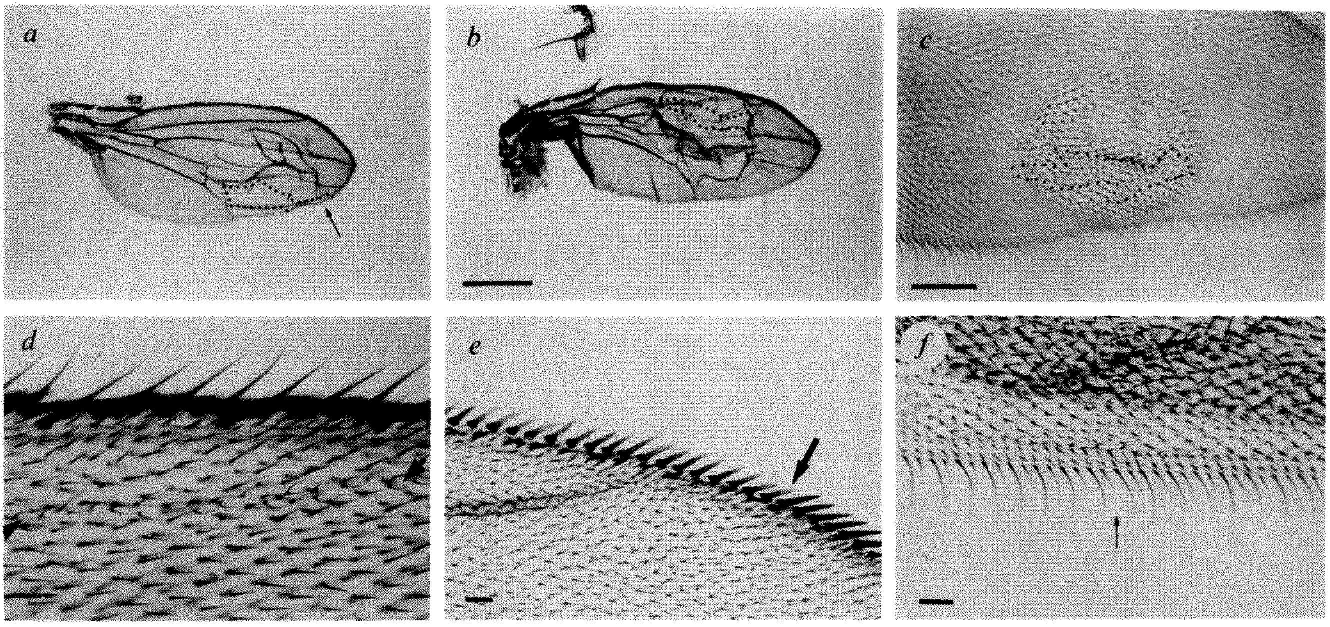

FIG. 3 Homozygous mys^{XG43} clones in adult wings. All clones illustrated were generated by irradiation at 48 ± 4 h after egg laying, except for that shown in *c*, which was generated at 72 ± 4 h. *a*, *b*, Blisters caused by large dorsal (*a*) or ventral (*b*) clones. The approximate extent of the clones is indicated by the dotted outlines; the clone in (*a*) abuts the wing margin distally (arrow). Note that the blisters are much larger than the clones, and are roughly circular. *c*, Blister resulting from small clone. The smooth oval shape of the blister contrasts with the irregular shape of the clone (dotted line). *d*, Long thin clone, which results in a discrete fold in the wing (between arrows), in contrast to the blisters. *e*, Clone along the anterior margin. The arrow indicates the last *y f* marginal bristle of the clone; the darker bristles to the right are wild type. This clone includes 35 bristles of the central row, yet has no obvious effect on the morphology of the margin. Overall, the only margin phenotype that was sometimes observed was that of folding near the wing edge, but this seemed to correlate with the presence of nearby blisters, and was probably an artefact of the more general disruption. *f*, Part of a large dorsal clone that abuts the posterior margin. Long ventrally derived wild-type marginal hairs alternate with shorter dorsally derived *y f* hairs (arrow). This clone includes 69 marginal hairs, all dorsal, in addition to its causing a large wing blister. Scale bars: *a* and *b*, 500 μm; *c*, 100 μm; *d*–*f*, 10 μm.

METHODS. For clonal analysis, *y* $mys^{XG43}f^{36a}/FM7$ females were crossed to wild-type males. The progeny were grown in uncrowded, well-yeasted vials, and irradiated with X-rays (1,500 rads) at 48 or 72 ± 4 h after egg laying, all at 25 °C. As a control, the same cross was performed with the markers but without the mys^{XG43} allele. Embryos homozygous for the mys^{XG43} allele[25] make no immunologically detectable β-subunit (ref. 5, and R. Salatino, unpublished results), and mys^{XG43} behaves as a typical strong allele in various complementation tests (R. Salatino and D.B., unpublished results); we therefore infer that it is a null allele of *mys*. On the basis of sizes and frequencies of mys^{XG43} and control clones, there was no indication that mys^{XG43} reduced cell proliferation. Initially, 133 wings were scored for marked clones without regard to overall wing morphology. These wings yielded 44 mys^{XG43} clones of greater than 50 cells, and virtually all of these clones were associated with clear morphological defects. Subsequently, we examined additional wings selected on the basis of abnormal morphology. Ultimately, a total of 135 wing clones were scored, about half of which included more than 100 cells. Although all results reported here are for the mys^{XG43} allele, similar wing blisters have been observed in clones of another putative null allele, mys^{XB87} (our unpublished observation).

derived central row of the anterior margin, but which did not cross to the other wing surface. These clones apparently reached the presumptive margin after the lineage restriction was established; even so, clones that define the margin over such distances would not be expected if the integrins are important for the maintenance of the lineage restriction.

Although most third instar imaginal disc cells express some surface PS integrin, these proteins are found in particularly high concentrations on the basal surface of the wing pouch epithelium[14]. Also, the spatial specificity of the PS1 and PS2 integrins in this disc region indicates that they are likely to be involved in a specific morphogenetic process there, and we have shown that the integrins are indeed critically important for the proper joining of the dorsal and ventral wing epithelia. It is interesting that our *mys* clones in the leg (15–30 bristles) or abdomen (7–10 bristles) displayed no obvious morphological phenotype (unpublished; see also ref. 21). It seems that the integrins serve a general function in discs, perhaps related to epithelium-matrix connections, but that they additionally perform a much more specialized morphogenetic function in the developing wing. ☐

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1. Brower, D. L., Wilcox, M., Piovant, M., Smith, R. J. & Reger, L. A. *Proc. natn. Acad. Sci. U.S.A.* **81**, 7485–7489 (1984).
2. Leptin, M., Aebersold, R. & Wilcox, M. *EMBO J.* **6**, 1037–1043 (1987).
3. Bogaert, T., Brown, N. & Wilcox, M. *Cell* **51**, 929–940 (1988).
4. MacKrell, A. J., Blumberg, B., Haynes, S. R. & Fessler, J. H. *Proc. natn. Acad. Sci. U.S.A.* **85**, 2633–2637 (1988).
5. Leptin, M., Bogaert, T., Lehmann, R. & Wilcox, M. *Cell* **56**, 401–408 (1989).
6. Hynes, R. O. *Cell* **48**, 549–554 (1987).
7. Ruoslahti, E. & Pierschbacher, M. D. *Science* **238**, 491–497 (1987).
8. Wilcox, M., Brown, N., Piovant, M., Smith, R. J. & White, R. A. H. *EMBO J.* **3**, 2307–2313 (1984).
9. Waddington, C. H. *J. Genet.* **41**, 75–139 (1941).
10. Bryant, P. J. *Devl Biol.* **22**, 389–411 (1970).
11. Garcia-Bellido, A. & Merriam, J. R. *Devl Biol.* **24**, 61–87 (1971).
12. Garcia-Bellido, A., Ripoll, P. & Morata, D. *Devl Biol.* **48**, 132–147 (1976).
13. Lindsley, D. L. & Grell, E. H. *Genetic Variations of* Drosophila melanogaster (Carnegie Institution of Washington Pub. No. 627, 1968).
14. Brower, D. L., Piovant, M. & Reger, L. A. *Devl Biol.* **108**, 120–130 (1985).
15. Postlethwait, J. H. in *Genetics and Biology of* Drosophila, Vol. 2c (eds Ashburner, M. & Wright, T. R. F.) 359–441 (Academic, London, 1978).
16. Wright, T. R. F. *J. exp. Zool.* **143**, 77–99 (1960).
17. Newman, S. M. & Wright, T. R. F. *Devl Biol.* **86**, 393–402 (1981).
18. Milner, M. J. & Muir, J. *Wilhelm Roux Arch. dev. Biol.* **196**, 191–201 (1987).
19. Burridge, K., Fath, K., Kelly, T., Nuckolls, G. & Turner, C. *A. Rev. Cell Biol.* **4**, 487–525 (1988).
20. Brower, D. L. *Cell* **41**, 361–364 (1985).
21. Wieschaus, E. & Noell, E. *Wilhelm Roux Arch. dev. Biol.* **195**, 63–73 (1986).
22. Bryant, P. J. in *Genetics and Biology of* Drosophila, Vol. 2c (eds Ashburner, M. & Wright, T. R. F.) 229–335 (Academic, London, 1978)
23. Carlson, P. S. *Genet. Res.* **17**, 53–81 (1971).
24. Falk, D. R., Roselli, L., Curtiss, S., Halladay, D. & Klufas, C. *Mutat. Res.* **126**, 25–34 (1984).
25. Wieschaus, E., Nüsslein-Volhard, C. & Jurgens, G. *Wilhelm Roux Arch. dev. Biol.* **193**, 296–307 (1984).

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Requirement of the *Drosophila raf* homologue for *torso* function

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In *Drosophila* the correct formation of the most anterior and posterior regions of the larva, acron and telson is dependent on the maternally expressed terminal class of genes[1]. In their absence, the anterior head skeleton is truncated and all the structures posterior to the abdominal segment seven are not formed[1–6]. The protein predicted to be encoded by one of these genes, *torso* (*tor*), seems to be a transmembrane protein with an extracytoplasmic domain acting as a receptor and a cytoplasmic domain containing tyrosine kinase activity[7]. Here we report that another member of the terminal-genes class, *l*(1)*polehole* (*l*(1)*ph*)[2,3,8], which is also zygotically expressed, is the *Drosophila* homologue of the v-*raf* oncogene and encodes a potential serine-and-threonine kinase. We also show that functional *l*(1)*ph* gene product is required for the expression of a gain-of-function *tor* mutant phenotype, indicating that *l*(1)*ph* acts downstream of *tor*. Together, these results support the idea that the induction of terminal development occurs through a signal transduction system, involving the local activation of the *tor*-encoded tyrosine kinase at the anterior and posterior egg poles, resulting in the phosphorylation of the *l*(1)*ph* gene product. In turn, downstream target proteins may be phosphorylated, ultimately leading to the regionalized expression of zygotic target genes. Such a process is in agreement with the finding that both *tor* and *l*(1)*ph* messenger RNAs are evenly distributed.

It has been shown previously that the gene encoding a potential serine-and-threonine kinase with homology to the v-*raf* oncogene[9–11] maps near the *l*(1)*ph* locus[12,13]. Molecular analysis of the DNA lesions associated with *l*(1)*ph* mutations indicates that the *Drosophila raf* homologue, *D-raf*, is in fact the *l*(1)*ph* gene (Fig. 1). Confirmation of the identity of *D-raf* and *l*(1)*ph* was obtained by P-element-mediated rescue experiments. A DNA fragment of 4.3 kilobases (kb) containing only the *D-raf* coding sequences rescued both the zygotic and maternal effects of *l*(1)*ph* mutations (Fig. 1*b*).

The *D-raf* transcripts are distributed homogeneously

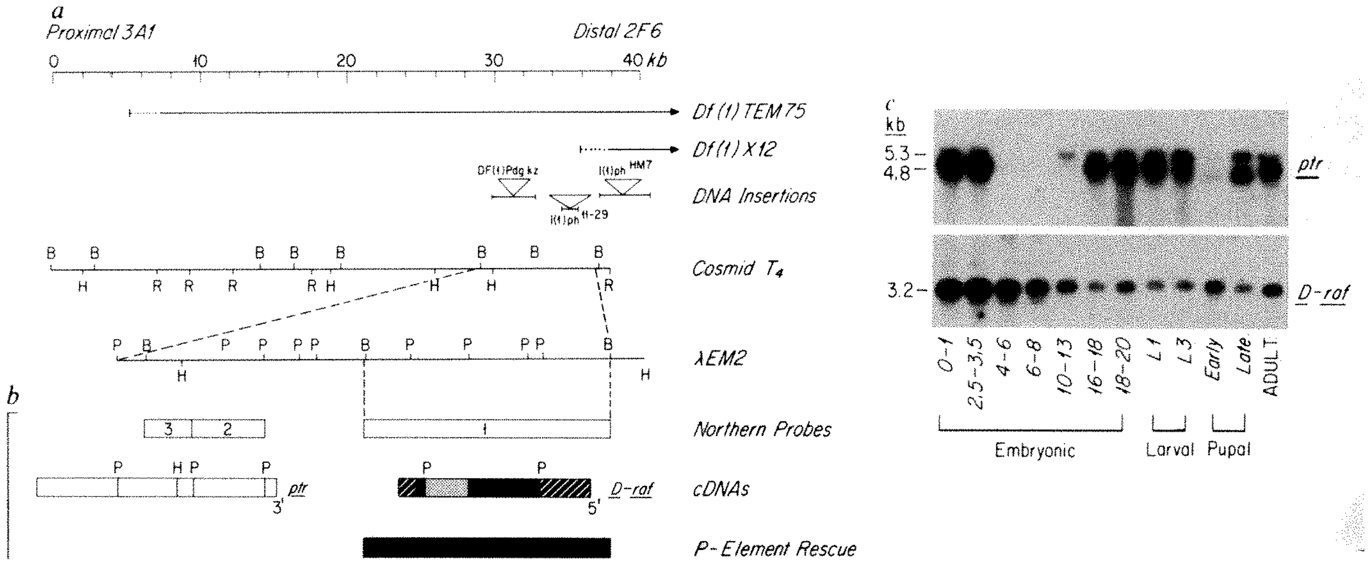

FIG. 1 The molecular organization of the 2F6-3A1 cytogenetic region containing the *l*(1)*ph* locus. The reference bar is graduated in kb with the position 0 defined by the proximal end of the T4 cosmid[23]. *a*, The *l*(1)*ph* gene lies between deficiency breakpoints *Df(1)TEM75* and *Df(1)X12*[8] which map to positions 6–8 and 36–38, respectively. The solid lines represent DNA sequences not removed by the deficiencies and the broken lines represent the maximum limits within which the deficiency breakpoints fall. *Df(1)TEM75* complements *l*(1)*ph* mutations, defining a proximal limit for the *l*(1)*ph* gene. The *Df(1)X12* chromosome does not complement *l*(1)*ph* mutations, indicating at least some of the *l*(1)*ph* gene lies proximal to the breakpoint at position 36–38. DNA polymorphisms in three different strains are identified as insertions in the *l*(1)*ph* region. Two mutant alleles of *l*(1)*ph*, $l(1)ph^{HM7}$ and $l(1)ph^{11-29}$ (L.A., manuscript in preparation) have ~4-kb DNA insertions mapping to positions 38–41 and 35–36, respectively. The *Df*(1)*Pdg kz* chromosome has wild-type *l*(1)*ph* activity but contains a 7-kb DNA insertion at position 30–33. These DNA insertions are depicted at their approximate positions by the inverted triangles. The deficiency breakpoints and DNA insertions were mapped using standard methods as described previously[24]. *b*, Two non-overlapping transcription units lie within the genomic region containing the *l*(1)*ph* gene. The *D-raf* gene was localized to position 33–37 using a *Bam*H1 subclone (nothern-blot probe 1) of the genomic probe *D-raf1* (ref. 12). A second transcription unit called *ptr* (proximal to *raf*) was identified by northern-blot analysis using probes 2 and 3. No additional bona fide transcription units are encoded within the DNA spanning positions 0–40. Complementary DNAs homologous to *D-raf* and *ptr* transcription units were isolated from λgt11 (gift of K. Zinn and C. Goodman) and plasmid pNB50 (ref. 25) cDNA libraries, respectively. The 3.2-kb *D-raf* cDNA contains both 700 base pairs-5′ and 600 base pairs-3′ nontranslated regions (hatched boxes), and a coding region of 1.9 kb (shaded boxes) which includes the putative serine-threonine kinase domain (vertical striped box). The 4-kb insertion in $l(1)ph^{11-29}$ maps within the *D-raf* coding region, whereas the $l(1)ph^{HM7}$ insertion maps 5′ to it. The complete structure of the 4.3 kb *ptr* cDNA has not been determined; but the *Df*(1)*Pdg kz* DNA insertion occurs within sequences encoding the *ptr* transcript. *Df*(1)*Pdg kz*/*Df*(1)*X12* females show only truncated *ptr* transcripts, have the normal 3.2 kb *D-raf* transcript, and are viable, fertile adults (data not shown). These data indicate that it is the *D-raf* rather than the *ptr* transcription unit which encodes the *l*(1)*ph* gene. A transformed strain, B-13-1 (ref. 13) containing a P-element construct carrying the wild-type *D-raf* gene was tested for complementation to *l*(1)*ph* mutations. All pleiotropic aspects of the mutant *l*(1)*ph* phenotype including the maternal effect, were rescued by the transformed DNA fragment *in trans* configuration (data not shown). *c*, Accumulation of *ptr* and *D-raf* transcripts during development. The *ptr* transcription unit encodes a 5.3 kb and a 4.3 kb RNA species and its expression is developmentally regulated. The 3.2 kb *D-raf* transcript is present at all stages of development. The time points for the embryonic period are given as hours after egg laying. These northerns blots re-probed with an actin 5C probe indicate that each lane contains an equivalent concentration of RNA (data not shown). Preparation of poly(A)$^+$ RNA and northern-blot analysis were performed as described previously[26].

throughout the oocyte and embryo at all stages (Fig. 2) and are not preferentially localized to the termini. Based on evidence presented below, it is likely that the maternal *D-raf*-encoded protein is also evenly distributed in the embryo. Similarly, there is no specific pattern to the localization of *tor* mRNA in the egg or embryo, and a uniform distribution of *tor* gene product is also predicted[7]. Thus the spatial restriction of both *D-raf* and *tor* activities, and hence the proper determination of terminal cell fates, does not rely on the spatial localization of the corresponding gene products, but could rather depend on their localized activation, possibly by one of the other terminal genes.

The finding that the proteins encoded by *D-raf* and *tor* contain domains with potential kinase activities indicates a possible mechanism by which such restricted activity of evenly distributed gene products occurs. In mammalian cells, the proto-oncogene homologue of v-*raf*, *c-raf-1* (refs 14, 15) encodes a protein which functions as a transducer of extracellular growth signals. Binding of growth factors, such as epidermal growth factor (EGF) and platelet-derived growth factor (PDGF) to receptors with tyrosine kinase activity causes the phosphorylation of *c-raf-1* product and the stimulation of its kinase activity[16]. Activated *c-raf-1* protein in turn phosphorylates other proteins, which may include itself and transcription factors, to initiate the programme for cell division. The proteins encoded by *tor* and *D-raf* could function in an analogous signal transduction pathway. The *tor* gene product could be the receptor for a spatially localized 'terminal' signal potentially secreted by the follicle cells during oogenesis. Binding of the localized ligand to the extracellular receptor domain of the *tor* gene product would activate the kinase domain, which would then locally phosphorylate the ubiquitous *D-raf* gene product. Thus, local activation of the *D-raf* gene product would lead to the induction of a programme of zygotic gene expression specific for the termini of the embryo.

We tested genetically for the interactions between *tor* and *D-raf* which such a model predicts. Loss-of-function alleles of *tor* lead to the loss of terminal structures identical to those described for null *l*(1)*ph* mutations. The maternal effect of some *tor* alleles, however, produce an opposite, gain-of-function embryonic phenotype[17,18]. Females homozygous for the *tor* gain-of function allele tor^{RL3} produce at 25 °C embryos that lack thoracic and abdominal structures but show differentiation of the terminal anlagen. Loss of abdominal segments is correlated with the suppression of early gap and segmentation gene expression, indicating that tor^{RL3} could encode a protein which is ectopically active in the central domain of tor^{RL3}-derived embryos[17,18].

If the *D-raf* gene product is required to transfer the activated *tor* signal to other downstream terminal genes, then the absence of *D-raf* should suppress the tor^{RL3} gain-of-function phenotype. To test whether this occurs, embryos derived from eggs lacking maternal *D-raf* and containing tor^{RL3} activities were examined. Two classes of embryos were observed with equal frequency after 24 hours of development at either 18 °C or 25 °C. Class 1 embryos showed the terminal class defect, with anterior and posterior regions of the embryos failing to develop. These embryos most probably represent the progeny lacking maternal *D-raf* activity. Class 2 embryos suffered massive cell death and developed little cuticle. These embryos are equivalent to those lacking both maternal and zygotic *D-raf* activity. No embryos exhibited the tor^{RL3} phenotype. Thus, functional *D-raf* gene product is required for expression of the gain-of-function tor^{RL3} phenotype. Loss of *D-raf* gene product blocks the transfer of the *tor* terminal signal in the central domain and at the termini of tor^{RL3}-derived embryos. This result also indicates that *D-raf* gene product is present and can be activated in the central domain of tor^{RL3} and wild-type embryos, which is consistent with the ubiquitous presence of *D-raf* transcripts (Fig. 2).

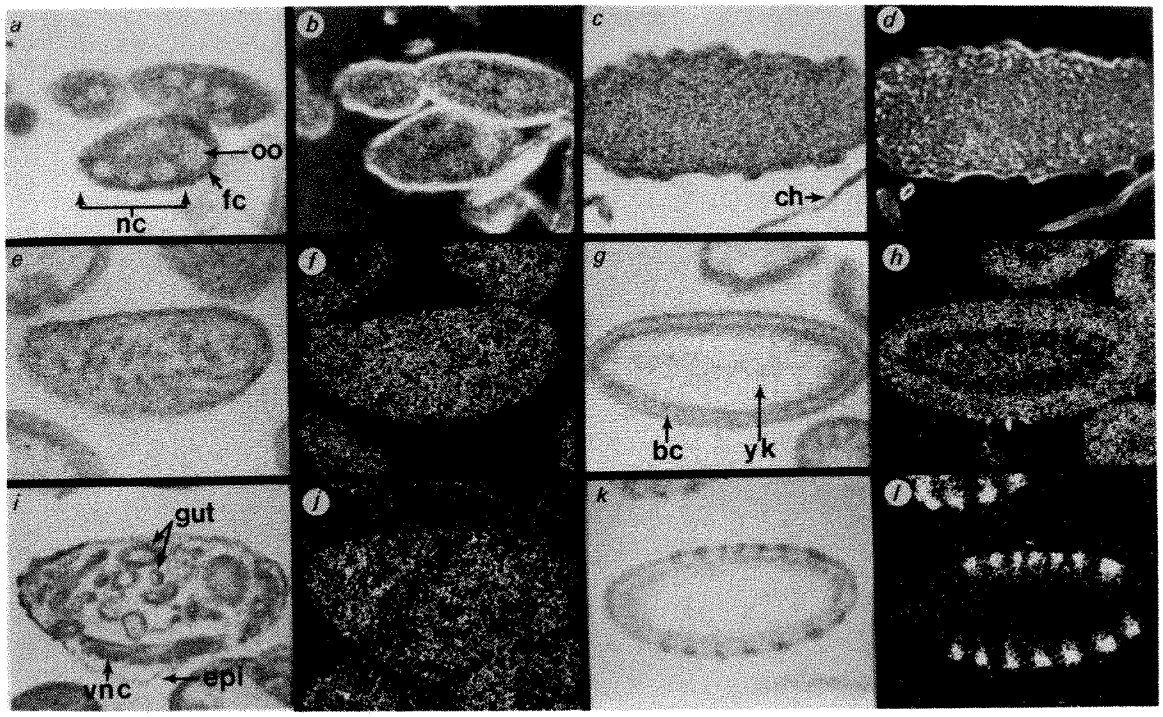

FIG. 2 Accumulation of *D-raf* transcripts during oogenesis and embryogenesis. *a, b*, An equal distribution of *D-raf* transcripts is observed for germ-line-derived nurse cells, oocyte and somatic follicle cells at stages 5–9 of oogenesis. *c, d*, A mature stage-14 oocyte shows no spatial localization of maternal *D-raf* RNAs. *e, f*, Accumulation of *D-raf* RNA in a syncitial blasoderm staged embryo 2 h after fertilization. *g, h*, At 3 h, *D-raf* RNA is present in the peripheral blastoderm cells and in the internal yolk region. *i, j*, At later stages of embryogenesis (16 h) *D-raf* transcripts are present in endodermal, mesodermal and ectodermal tissues. *k, l*, Control blastoderm stage embryo showing localization of *fushi tarazu* (*ftz*) transcripts[27]. Preparation of tissue sections and hybridization conditions using ^{35}S-labelled *D-raf* and *ftz* probes were as described previously[26]. Abbreviations: bc, blastoderm cells; ch, chorion; epi, epidermis; fc, follicle cells; nc, nurse cell complex; oo, oocyte; vnc, ventral nerve cord; yk, yolk.

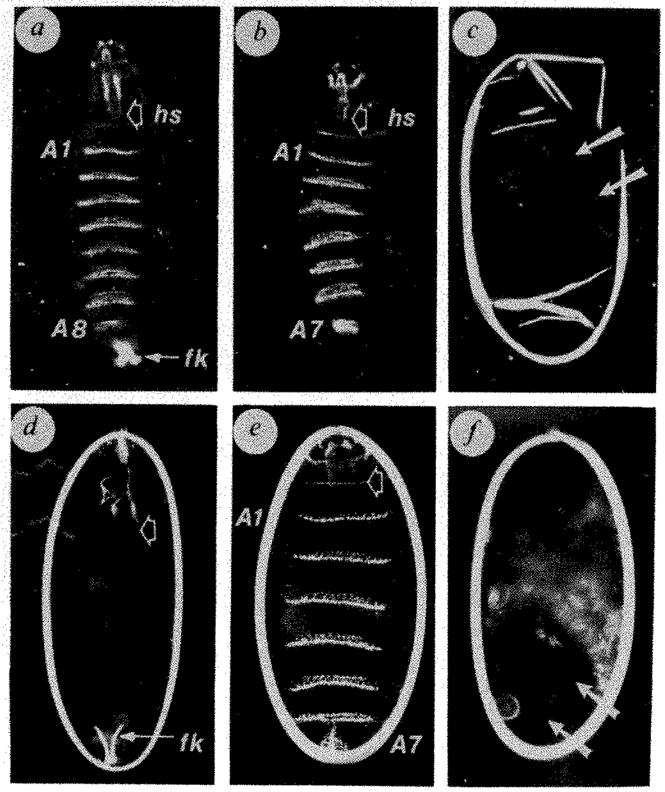

FIG. 3 The phenotype of embryos derived from double mutant *l*(1)*ph* and tor^{RL3} female germ cells. Dark field photographs of cuticular preparations of wild-type embryos (*a*) and of embryos derived from: homozygous *l*(1)*ph* female germ cells (*b*, *c*); homozygous tor^{RL3} females (*d*); homozygous *l*(1)*ph*;tor^{RL3} female germ cells (*e*, *f*). In *b* and *e*, embryos show the terminal class phenotype: a truncated head skeleton and the lack of structures posterior to abdominal segment seven. These embryos developed without maternal *D-raf* activity. In *c* and *f*, embryos show the *l*(1)*ph* 'null' phenotype; little cuticle is formed. These embryos developed without maternal and zygotic *D-raf* activity. *d*, The tor^{RL3} gain-of-function phenotye: at 19 °C embryos form head and tail structures without thoracic or abdominal segments. The $l(1)ph^{EA75}$ allele used to generate female germ cells lacking *D-raf* activity as previously described[28]. All females were crossed to Oregon R wild-type males. Abbreviations: fk, filzkorper; hs, head skeleton.

TABLE 1 Partial suppression of the tor^{RL3}

Maternal genotype	*T* (°C)	*N*	No. of embryos hatched	Hatching (%)
A. tor^{RL3}/tor^{RL3}	18	1,920	20	1
	19	2,812	0	0
	20	1,882	0	0
B. *l*(1)*ph*/*FM3* tor^{RL3}/tor^{RL3}	18	1,906	220	11.5
	19	2,788	110	3.9
	20	1,320	3	0.22
C. ±/*FM3*: tor^{RL3}/tor^{RL3}	18	1,950	30	1.5
	19	1,478	11	0.7
	20	1,520	0	0

The frequency of embryonic hatching was assayed 48 h after egg laying; females were mated to Oregon R wild-type males. *A*, Eggs produced by tor^{RL3}/tor^{RL3} females collected from the original stock obtained from T. Schupbach. For *B* and *C* the following stock was made: $l(1)ph/FM3/Dp(1;Y)w^{+303};tor^{RL3}/CyO$. From this stock *l*(1)*ph*/*FM3*; tor^{RL3}/tor^{RL3} females were collected and assayed in *B*. For the females assayed in *C*, *l*(1)*ph*/*FM3*; tor^{RL3}/CyO females were mated to tor^{RL3}/CyO males and the ±/*FM3*; tor^{RL3}/tor^{RL3} females collected. In this experiment, surviving larvae were phenotypically normal and some developed into adults. The unhatched embryos had segmentation defects which were more extreme at 20 °C than at 18 °C. A null allele, $l(1)ph^{EA75}$, was used in this study. Descriptions of balancer chromosomes, *FM3* and *CyO* in ref. 22.

Suppression of the gain-of-function tor^{RL3} phenotype is also observed in embryos derived from eggs containing only half the wild-type amount of *D-raf* gene product. At 18 °C, the proportion of embryos that hatched was greater for these embryos than for those with normal levels of *D-raf* activity (Table 1). The maternal effect of tor^{RL3} is cold sensitive[18]; at 25 °C, embryos show complete loss of abdominal segmentation, whereas at 18 °C a few embryos (1%) have normal segmentation and hatch. This weak gain-of-function phenotype of the tor^{RL3} allele at 18 °C is indicative of a low-level of ectopic *tor* activity. But when the maternal contribution of functional *D-raf* gene product was reduced by half there was a 10-fold increase (11%) in the number of embryos that survive to hatching. The enhanced survival can be attributed to the reduced levels of maternal *D-raf* activity, and, thus, to an indirect reduction of ectopic tor^{RL3} activity. When the availability of *D-raf* gene product is reduced, the efficiency of signal transfer may also decrease, thereby restoring normal embryonic development. At the termini, where the activity of tor^{RL3} is at least normal, a 50% reduction of *D-raf* product does not adversely affect the development of head or tail structures.

These results support the idea that induction of terminal development acts through a signal transduction system involving a phosphorylation cascade. Spatial restriction of activity is achieved in this pathway by the localized activation of evenly distributed gene products. Two genes likely to participate in this cascade are *tor* and *l*(1)*ph* (*D-raf*). We have shown here that *D-raf* acts downstream of the *tor* gene product to transfer the maternal terminal signal. Our data is consistent with the model proposed above and by Sprenger *et al.*[7]: activation of *tor*-encoded tyrosine kinase in a spatially restricted manner results in the phosphorylation of *D-raf* gene product, which in turn phosphorylates serines and threonines of downstream target proteins. One such target could be the protein encoded by the *tailless* (*tll*) gene[19]; phosphorylation by *D-raf* gene product of maternally expressed Tll protein[18] could cause its activation, leading directly or indirectly to zygotic expression of *tll*. Other downstream terminal genes, including *fork head*[20] and *spalt*[21] could play a part in mediating the terminal class signal transmitted by *D-raf*.

In contrast to the other maternal terminal class genes, *l*(1)*ph* gene activity is also required at later embryonic and larval stages of development for the maintenance of cell viability[3] and for the proliferation of somatic cells[2], respectively. Thus the *l*(1)*ph* gene product acts in several different developmental pathways; only its maternal role in the terminal class pathway has been addressed here. ☐

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1. Nüsslein-Volhard, C., Frohnhofer, H., G. & Lehmann, R. *Science* **238**, 1675–1681 (1987).
2. Perrimon, N., Engstrom, L. & Mahowald, A. P. *Devl Biol.* **110**, 480–491 (1985).
3. Ambrosio, L., Mahowald, A. P. & Perrimon, N. *Development* **106**, 145–158 (1989).
4. Degelmann, A., Hardy, P. A., Perrimon, N. & Mahowald, A. P. *Devl Biol.* **115**, 479–489 (1986).
5. Perrimon, N., Mohler, D., Engstrom, L. & Mahowald, A. P. *Genetics* **113**, 695–712 (1986).
6. Schüpbach, T. & Wieschaus, E. *Wilhem Roux Arch. dev. Biol.* **195**, 302–317 (1986).
7. Sprenger, F., Stevens, L. M. & Nusslein-Volhard, C. *Nature* **338**, 478–483 (1989).
8. Perrimon, N., Engstrom, L. & Mahowald, A. P. *Genetics* **108**, 559–572 (1984).
9. Rapp, U. R. *et al. Proc. natn. Acad. Sci. U.S.A.* **80**, 4218–4222 (1983).
10. Jansen, H. *et al. Nature* **307**, 281–284 (1984).
11. Moelling, K., Heimann, B., Beimling, P., Rapp, U. R. & Saunders, T. *Nature* **312**, 558–561 (1984).
12. Mark, G. E., Macintyre, R. J., Digan, M. E., Ambrosio, L. & Perrimon, N. *Molec. cell. Biol.* **7**, 2134–2140 (1987).
13. Nishida, Y. *et al. EMBO J.* **7**, 775–781 (1988).
14. Bonner, T. I. *et al. Nucleic Acids Res.* **14**, 1009–1015 (1986).
15. Rapp, U. R., Cleveland, J. L., Storm, S. M., Beck, T. W. & Huleihel, M. in *Oncogenes and Cancer* (ed. Aaronson, S. A.) 55–74 (Japan Sci. Soc., Tokyo, 1987).
16. Morrison, D. K., Kaplan, D. R., Rapp, U. R. & Roberts, T. M. *Proc. natn. Acad. Sci. U.S.A.* **85**, 8855–8859 (1988).
17. Klinger, M., Erdelyi, M., Szabad, J. & Nüsslein-Volhard, C. *Nature* **335**, 275–277 (1988).
18. Strecker, T. R., Halsell, S. R., Fisher, W. W. & Lipshitz, H. D. *Science* **243**, 1062–1066 (1988).
19. Strecker, T. R., Merriam, J. R. & Lengyel, J. A. *Development* **102**, 721–734 (1988).
20. Weigel, D., Jürgens, G., Kuttner, F., Seifert, E. & Jackle, H. *Cell* **57**, 645–658 (1989).
21. Frei, E. *et al. EMBO J.* **7**, 197–204 (1988).
22. Lindsley, D. L. & Grell, E. H. in *Genetic variations of* Drosophila melanogaster (Carnegie Institution of Washington. Publication No. 627, 1968).

23. Mariani, C., Pirrotta, V. & Manet, E. *EMBO J.* **4**, 2045–2052 (1985).
24. Maniatis, T., Fritsch, E. F. & Sambrook, J. in *Molecular Cloning: a Laboratory Manual* (Cold Spring Harbor Press, New York, 1982).
25. Brown, N. H. & Kafatos, F. C. *J. molec. Biol.* **203**, 425–437 (1988).
26. Shi-Chung N. G., Perkins, L. A., Conboy, G., Perrimon, N. & Fishman, M. C., *Development* **105**, 629–638 (1989).
27. Hafen, E., Kuroiwa, A. & Gehring, W. J. *Cell* **37**, 833–841 (1984).
28. Perrimon, N. *Genetics* **108**, 927–939 (1984).

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Substantial increase of protein stability by multiple disulphide bonds

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DISULPHIDE bonds can significantly stabilize the native structures of proteins[1–3]. The effect is presumed to be due mainly to a decrease in the configurational chain entropy of the unfolded polypeptide[4–7]. In phage T4 lysozyme, a disulphide-free enzyme, engineered disulphide mutants that crosslink residues 3–97, 9–164 and 21–142 are significantly more stable than the wild-type protein[8–11]. To investigate the effect of multiple-disulphide bonds on protein stability, mutants were constructed in which two or three stabilizing disulphide bridges were combined in the same protein. Reversible thermal denaturation shows that the increase in melting temperature resulting from the individual disulphide bonds is approximately additive. The triple-disulphide variant unfolds at a temperature 23.4 °C higher than wild-type lysozyme. The results demonstrate that a combination of disulphide bonds, each of which contributes to stability, can achieve substantial overall improvement in the stability of a protein.

Unpaired cysteine residue(s) in a protein containing a disulphide bond(s) can lead to oligomerization through thiol/disulphide interchange[12]. Previously described single-disulphide mutants (designated as 3C–54T, 9C–164C-wt* and 21C–142C-wt*, see Fig. 1) were, therefore, constructed in an otherwise cysteine-free pseudo wild-type lysozyme (wt*)[11,13]. For the same reason the two double-disulphide mutants D3–97/9–164 and D9–164/21–142 and the triple disulphide mutant T3–97/9–164/21–142 were also designed and constructed to have no unpaired cysteines. Figure 1 shows the locations of the disulphide bonds and ancillary mutations introduced into T4 lysozyme. Each of the mutant genes was expressed in *Escherichia coli* and the proteins purified to homogeneity. Immediately after purification, the mutant enzymes were found to be a mixture of oxidized (crosslinked) and reduced (noncrosslinked) forms, as judged by ion-exchange high-performance liquid chromatography (HPLC)[11], reversed-phase HPLC[8,13] and titration of protein thiols with Ellman's reagent (data not shown). After exposure of proteins to air under mild alkaline conditions (pH 8.0) for several days, however, the mutant proteins were all converted to the oxidized forms (Table 1). Nonreducing SDS-PAGE showed that the double- and triple-disulphide mutants migrate faster than the disulphide-free wild-type lysozyme, whereas all the multiple-disulphide mutants had mobility identical with wild type in the presence of reducing agent (data not shown). This observation indicates that the crosslinked proteins have a more compact structure in the denatured state.

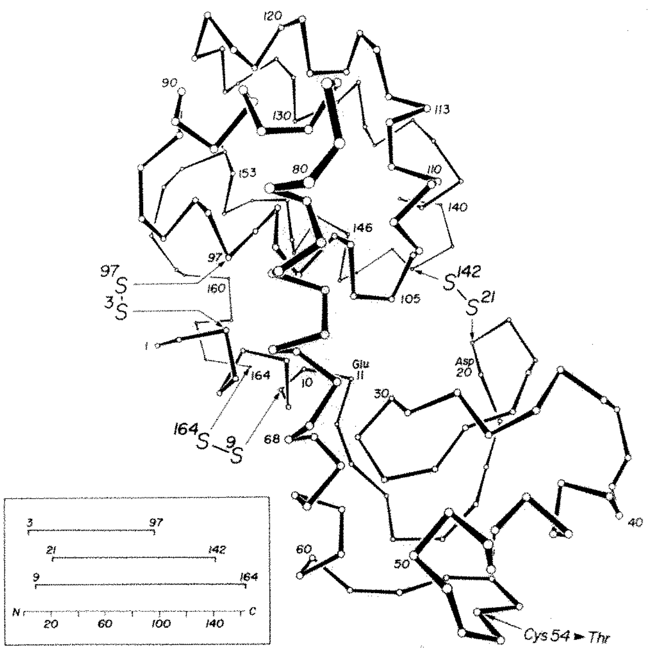

FIG. 1 Backbone of T4 lysozyme showing the locations of the three engineered disulphide bridges. The insert illustrates the loops formed by these bridges. Identification and generation of the mutant lysozymes is as follows:

Variant	Amino-acid replacements 3	9	21	54	97	142	164	No. of cysteines	No. of disulphide bonds
wt	Ile	Ile	Thr	Cys	Cys	Thr	Leu	2	0
wt*				Thr	Ala			0	0
3C–54T	Cys			Thr				2	1
9C–164C-wt*		Cys		Thr	Ala		Cys	2	1
21C–142C-wt*			Cys	Thr	Ala	Cys		2	1
D3–97/9–164	Cys	Cys		Thr			Cys	4	2
D9–164/21–142		Cys	Cys	Thr	Ala	Cys	Cys	4	2
T3–97/9–164/21–142	Cys	Cys	Cys	Thr		Cys	Cys	6	3

Recombinant DNA techniques used in the construction of the disulphide mutants were essentially as described[17]. Mutagenic oligonucleotides (21–23 mer) were synthesized using a model 380B DNA synthesizer (Applied Biosystems) and purified by a C18 Sep-Pak cartridge (Millipore). The single-stranded DNA template for site-directed mutagenesis was an M13mp18 derivative containing the T4 lysozyme gene on a 630 base-pair *Bam*HI–*Hind*III fragment. The mutagenesis was performed according to Kunkel *et al.*[18] using *E. coli* strain CJ236 (*dut1, ung1, thi1, relA1*/pCJ105 (Cm^r)). After the repair synthesis of DNA *E. coli* JM101 (ref. 19) was transformed and the mutants identified either by DNA sequencing[20] or plaque hybridization with the $[^{32}P]$-labelled mutagenic prime[21]. The mutations were verified by sequencing the entire T4 lysozyme gene. The mutated T4 lysozyme gene on M13 was digested with *Bam*HI and *Hind*III, and cloned into the expression plasmid pHSe5 that contains *tac* and *lac*UV5 promoters, $lacI^q$ gene as well as *trp* terminator[22]. *E. coli* RR1 (ref. 17) was then transformed by the recombinant pHSe5 and the mutant protein was overproduced by addition of isopropyl-β-thiogalactoside. The mutant proteins were purified to homogeneity by CM-Sepharose and SP-Sephadex (Pharmacia) chromatography as described[22].

The activity of the D3–97/9–164 mutant (Table 1) is indistinguishable from that of wild-type enzyme in both the oxidized and reduced forms. The result is consistent with the observation that the corresponding single-disulphide mutants have essentially the same activities as wt* in both oxidized and reduced forms[11]. In addition it indicates that virtually all the D3–97/9–164 molecules have the correct pairing of the disulphide bridges as any mispairing would presumably lead to a loss of activity. The wt* lysozyme loses enzymatic activity at ~55 °C at pH 7.4, whereas the D3–97/9–164 mutant, which is more thermostable than wt* by 15.7 °C at pH 2.0 (see below), retains activity up to

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TABLE 1 Properties of mutant lysozymes

Variant	Ox/Red	Thiols (mol SH/mol prot.)	Relative activity (%)	T_m (°C)	ΔT_m(mut-wt*) (°C)	ΔT_m(ox-red) (°C)
wt	Ox	1.9	(100)		—	—
	Red	2.0	100	41.9		
wt*	Ox	0.0	(103)			
	Red	0.0	103	41.9	0.0	0.0
3C/54T	Ox	ND	96	46.7	4.8	6.7
	Red	ND	90	40.0	−1.9	
9C/164C/wt*	Ox	ND	106	48.3	6.4	12.9
	Red	ND	99	35.4	−6.5	
21C/142C/wt*	Ox	ND	0	52.9	11.0	13.7
	Red	ND	68	39.2	−2.7	
D3-97/9-164	Ox	0.0	95	57.6	15.7	26.0
	Red	4.1	100	31.6	−10.3	
D9-164/21-142	Ox	0.0	0	58.9	17.0	22.9
	Red	4.1	58	36.0	−5.9	
T3-97/9-164/21-142	Ox	0.2	0	65.5	23.4	31.9
	Red	6.0	43	33.4	−8.5	

Oxidized (crosslinked) mutant lysozymes were prepared on exposure to air *in vitro* by incubating in 0.1 M Tris-HCl, 0.15 M NaCl (pH 8.0) for several days. The reduced (noncrosslinked) forms of the mutant lysozymes were prepared by treating the purified proteins (~1 mg ml^{-1}) with 6 M guanidine hydrochloride, 20 mM DTT and 1 mM EDTA in 50 mM Tris-HCl (pH 8.3). After incubation for 4 h at 23 °C, the mixture was extensively dialysed under a nitrogen gas purge at 4 °C against 0.2 M NaCl and 1 mM EDTA (pH 2.0). Under the nitrogen atmosphere and at acid pH, the reduced proteins were stable for at least a week at 4 °C. Thiol content was determined by Ellman titration[23]. A 0.5 ml fraction of the oxidized or reduced protein (0.02 μmole) was mixed with 2.5 ml of a solution containing 2% SDS, 0.08 M sodium phosphate (pH 8.0), and 0.5 mg ml^{-1} EDTA. To 3 ml of the solution was immediately added 0.1 ml 5,5-dithiobis (2-nitrobenzoic acid) (DTNB) (40 mg DTNB in 10 ml of 0.1 M sodium phosphate, pH 8.0). After 15 min, the absorbance at 410 nm was measured. Lysozyme activities were measured at 23 °C using the turbidity assay[24]. Protein concentrations were determined spectrophotometrically (molar absorption coefficient for wild-type T4 lysozyme $\epsilon^{0.1\%}_{280\,\mathrm{nm}}=1.28$) (ref. 24). T_m is the temperature at the midpoint of the thermal denaturation transition at pH 2.0. ΔT_m(mut-wt*) and ΔT_m(ox-red) are the differences between mutant and wt* lysozyme and between the oxidized and reduced mutant protein, respectively. Data for wild type and single S-S bridge lysozymes are from refs 10, 11 and 25. Thermal stability was assessed at pH 2.0 by measuring the circular dichroism at 223 nm as a function of temperature[26,27]. Solutions contained ~0.02 mg ml^{-1} protein, 0.15 M KCl and 1 mM EDTA, adjusted to pH 2.0 with HCl. To avoid air oxidation, the experiments were performed in a nitrogen atmosphere and the KCl-EDTA solutions were extensively bubbled with nitrogen gas before use[28].

~65 °C (data not shown). Mutants D9-164/21-142 and T3-97/9-164/21-142, which include the 21-142 disulphide bond, have virtually no catalytic activity in the oxidized form. This is expected as the 21-142 disulphide bond spans the 'mouth' of the active-site cleft (Fig. 1) leading to the complete abolition of lysozyme activity[13].

The melting temperatures of the single and multiple disulphide bridge mutants at pH 2.0 are summarized in Table 1 and Fig. 2. Both the double mutants are more heat-stable than their constitutent single mutants. In the case of the triple mutant the increase in melting temperature (ΔT_m) is substantial (23.4 °C) and agrees surprisingly well with the sum of the ΔT_ms of the corresponding single disulphide variants (22.2 °C). Measurement of the melting temperature at higher pH values is difficult because the solutions approach boiling point before the unfolding transition is complete. As a guide, however, the estimated reversible melting temperature of the triple disulphide mutant at pH 5.0 is 86 °C, which is 20 °C higher than wild type at the same pH.

It is presumed that a consequence of a disulphide bridge is to reduce the configurational backbone chain entropy of the protein in the denatured state, thus increasing the stability of the folded protein[3-7]. Theoretical considerations suggest that the entropic contribution to the free energy of stabilization increases in proportion to the logarithm of the number of residues in the loop formed by the disulphide bridge[3-6]. When multiple, overlapping disulphide bonds are introduced into a protein, their effects on the configuration of the unfolded protein are interdependent, and the determination of the entropic term becomes more complicated[6,7,14]. Nevertheless, reasonable agreement between experiment and theory has been observed for ribonuclease A[6,14], ribonuclease T1 (ref. 3) and hen egg-white lysozyme[15]. Based on theory,[14] the reductions in entropy in the unfolded protein caused by the single 3-97, 9-164 and 21-142 bridges are, respectively, 20.5, 21.9 and 21.2 entropy units. The sum of these three terms, considered independently, is 63.6 entropy units, which corresponds to 20.0 kcal mol^{-1} at 42 °C, the melting temperature of wild-type lysozyme at pH 2.0. By contrast, the theoretically estimated[14] entropy reduction when all three bridges are included in the same protein is 56.7 entropy units, corresponding to 17.9 kcal mol^{-1}. Thus, even though the loops formed by the three disulphide bridges have substantial overlap (Fig. 1), this has only a relatively small effect on the

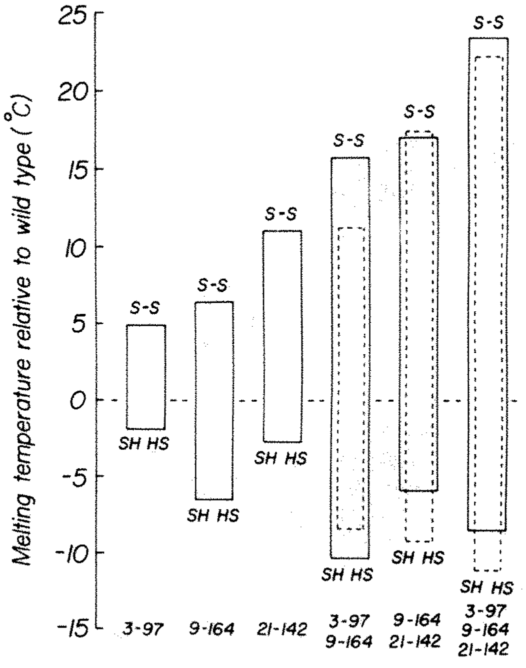

FIG. 2 Melting temperatures of single, double and triple-disulphide bonded lysozymes relative to wild-type lysozyme (ΔT_m) at pH 2.0. The solid bars show the observed melting temperatures of the oxidized and reduced forms of the mutant lysozymes (Table 1). The broken bars for the multiply bridged proteins correspond to the sums of the ΔT_ms for the constitutent singly bridged lysozymes.

overall entropy term. Although each disulphide bridge reduces the conformational freedom of the unfolded chain, this restriction represents only a small fraction of the total number of conformational states that are available. Thus, the contributions to stability from each of the three disulphide bridges are still largely additive. The crosslinked lysozymes display a correlation between the number of disulphide bonds and the Stokes radii of the denatured proteins, as judged by gel filtration HPLC in the presence of urea[16]. This also indicates that the crosslinks successively decrease the configurational freedom of the unfolded protein.

The above arguments are based on entropy considerations alone. As discussed in ref. 11, the net stabilization of a given disulphide bond depends on other factors, such as the loss of stabilizing interactions associated with introduction of the cysteine(s) and possible introduction of strain associated with the closure of the disulphide bridge. In the case of T4 lysozyme, factors that seem to be helpful in the design of the stabilizing disulphide bridges include the use of large loops to maximize the entropic effect on the unfolded state and the choice of flexible sites to avoid the introduction of strain into the folded protein[11]. Exploration of the energetic and theoretical basis of the observed stabilization of the multiply bridged lysozymes will require detailed thermodynamic analysis of these proteins.

In terms of the goal of developing general methods to increase the stability of proteins, the present results are especially encouraging. They demonstrate that individual S–S bridges can be combined together to dramatically enhance stability, even in cases where the disulphide bridges form overlapping loops. It is also encouraging to find that the double-disulphide mutant D3-97/9-164 is fully active and also retains activity at temperatures substantially higher than wild-type lysozyme. This shows that a protein does not have to be marginally stable in order to be fully active. Instead, the present results indicate that the stability of a protein can be substantially increased with no loss in enzymatic activity. □

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1. Creighton, T. E. *BioEssays* **8**, 57–63 (1988).
2. Thornton, J. M. *J. molec. Biol.* **151**, 261–287 (1981).
3. Pace, C. N., Grimsley, G. R., Thomson, J. A. & Barnett, B. J. *J. biol. Chem.* **263**, 11820–11825 (1988).
4. Schellman, J. A. *C. R. Trav. Lab. Carlsberg Ser. Chim.* **29**, 230–259 (1955).
5. Flory, P. J. *J. Am. Chem. Soc.* **78**, 5222–5235 (1956).
6. Poland, D. C. & Scheraga, H. A. *Biopolymers* **3**, 379–399 (1965).
7. Chan, H. S. & Dill, K. A. *J. chem. Phys.* **90**, 492–509 (1988).
8. Perry, L. J. & Wetzel, R. *Science* **226**, 555–557 (1984).
9. Wetzel, R., Perry, L. J., Baase, W. A. & Becktel, W. J. *Proc. natn. Acad. Sci. U.S.A.* **85**, 401–405 (1988).
10. Matsumura, M., Becktel, W. J. & Matthews, B. W. *Nature* **334**, 406–410 (1988).
11. Matsumura, M., Becktel, W. J., Levitt, M. & Matthews, B. W. *Proc. natn. Acad. Sci. U.S.A.* **86**, 6562–6566 (1989).
12. Perry, L. J. & Wetzel, R. *Biochemistry* **25**, 733–739 (1986).
13. Matsumura, M. & Matthews, B. W. *Science* **243**, 792–794 (1989).
14. Lin, S. H., Konishi, Y., Denton, M. E. & Scheraga, H. A. *Biochemistry* **23**, 5504–5512 (1984).
15. Johnson, R. E., Adams, P. & Rupley, J. A. *Biochemistry* **17**, 1479–1484 (1978).
16. Signor, G., Matsumura, M., Schellman, J. A. & Matthews, B. W. in *Current Research in Protein Chemistry* (ed. Villafranca, J. J.) (Academic, in the press).
17. Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1982).
18. Kunkel, T. A., Roberts, J. D. & Zakour, R. A. *Meth. Enzym.* **154**, 367–382 (1987).
19. Messing, J. *Meth. Enzym.* **101**, 20–78 (1983).
20. Sanger, F., Nickelsen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74**, 5463–5467 (1977).
21. Zoller, M. J. & Smith, M. *Meth. Enzym.* **100**, 468–500 (1983).
22. Muchmore, D. C., McIntosh, L. P., Russell, C. B., Anderson, D. E. & Dahlquist, F. W. *Meth. Enzym.* **177**, 44–73 (1989).
23. Habeeb, A. F. S. A. *Meth. Enzym.* **25**, 457–464 (1972).
24. Tsugita, A., Inouye, M., Terzaghi, E. & Streisinger, G. *J. biol. Chem.* **243**, 391–397 (1968).
25. Matthews, B. W., Nicholson, H. & Becktel, W. J. *Proc. natn. Acad. Sci. U.S.A.* **84**, 6663–6667 (1987).
26. Elwell, M. & Schellman, *J. biochem. biophys. Acta* **386**, 309–323 (1975).
27. Hawkes, R., Grütter, M. G. & Schellman, J. *J. molec. Biol.* **175**, 195–212 (1984).
28. Becktel, W. J. & Baase, W. A. *Biopolymers* **26**, 619–623 (1987).

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Focal points for chromosome condensation and decondensation revealed by three-dimensional *in vivo* time-lapse microscopy

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ALTHOUGH the dynamic behaviour of chromosomes has been extensively studied in their condensed state during mitosis, chromosome behaviour during the transition to and from interphase has not been well documented. Previous electron microscopic studies suggest that chromosomes condense in a non-uniform fashion at the nuclear periphery[1,2]. But chromosome condensation is a complicated and dynamic process and requires continuous observation in living tissues to be fully understood. Using a recently developed three-dimensional time-lapse fluorescence microscopy technique[3], we have observed chromosomes as they relax from telophase, through interphase, until their condensation at the next prophase. This technique has been improved to produce higher-resolution images by implementing new stereographic projection and computational processing protocols[4]. These studies have revealed that chromosomal regions on the nuclear envelope, distinct from the centromeres and telomeres, serve as foci for the decondensation and condensation of diploid chromosomes. The relative positions of the late decondensation sites at the beginning of interphase appear to correspond to the early condensation sites at the subsequent prophase.

In order to study the chromosomal events that occur between telophase and the ensuing prophase, we have followed embryonic nuclei of *Drosophila melanogaster* during the late syncytial blastoderm period. Chromosomes in living *Drosophila* embryos were visualized by imaging chromatin which had assembled fluorescent-labelled histones into nucleosomes. Calf thymus histones H2A and H2B were conjugated with rhodamine and microinjected into *Drosophila* embryos 30 minutes after oviposition to ensure a uniform distribution of the labelled histones by the time of observation. The injected rhodamine-labelled histones are stably incorporated into chromatin during periods of DNA replication. Under low levels of illumination, these embryos can be observed for more than three hours and develop normally to hatching.

Observing the behaviour of an entire nucleus requires a three-dimensional analysis over time. Imaging at low light levels was accomplished using a cooled, scientific grade charge-coupled device (CCD) imager[5,6] operated by a self-contained computer workstation. Three-dimensional data sets were obtained by digitally recording a series of five optical sections taken at 1 μm focal intervals every five seconds, as described in the legend to Fig. 1. By repeating this optical sectioning protocol every 25 seconds, a time-lapse series of 80 three-dimensional data sets was recorded. The period of analysis spanned nuclear cycles 12 and 13 of syncytial blastoderm stage embryos. During this stage of embryonic development, the nuclei form an almost synchronously replicating monolayer on the embryo surface[7,8]. The nuclear cycle length for the embryo shown in Fig. 1 was 22 minutes at 23 °C. Embryos were monitored after data collection to verify that gastrulation occurred normally.

To facilitate analysis of the large amount of three-dimensional data over time, a time-lapse stereo movie was constructed using the synthetic projection method[4] described in the legend to Fig. 1. Briefly, a stereo pair of projected images is formed from each set of optical section data by sequentially shifting each image either left or right and summing to generate the projections. The projected images are then computationally processed to remove out-of-focus information[4]. For projected images, this is a simple and straightforward process which can provide nearly exact deblurring. Furthermore, as no light is rejected in the image processing, unlike confocal microscopy, this approach is ideal for the low-light level imaging required for *in vivo* analysis. The resultant in-focus stereo projection images were sequentially displayed in rapid succession as a time-lapse movie. Examples of selected frames of the movie are shown as stereo pairs in Fig. 1.

Examination of the real-time, three-dimensional data revealed that chromosome decondensation terminates and condensation initiates at discrete sites predominantly on the nuclear envelope (visualized as bright spots; Fig. 2*a–e*). Chromosomes begin to decondense shortly after chromatid separation, and by the following interphase, nuclei labelled with rhodamine-conjugated histones show an essentially uniform appearance. Surprisingly, examination of the kinetics of chromosome decondensation revealed that a small portion of the chromosomes remained condensed at several sites on the nuclear envelope (2–3 sites per nucleus) after most of the chromosomes had decondensed (about 5 minutes after the metaphase–anaphase transition of the twelfth cycle). The location of these sites can be seen clearly at the nuclear periphery (indicated by arrows in Fig. 2*a–e*, column 1). Because these late decondensation sites eventually disappear to make a uniformly stained nucleus during interphase (about 6 minutes after the metaphase-anaphase transition; see Fig. 1, frame at 6:40 min), they are not consistent with the classical definition of heterochromatin.

At the transition from interphase to prophase, bright spots of chromosome condensation were also observed at discrete sites on the nuclear envelope (indicated by arrows in Fig. 2*a–e*, columns 3-6). The first of these condensation sites appears 10 minutes after nuclear division, with new sites continuing to

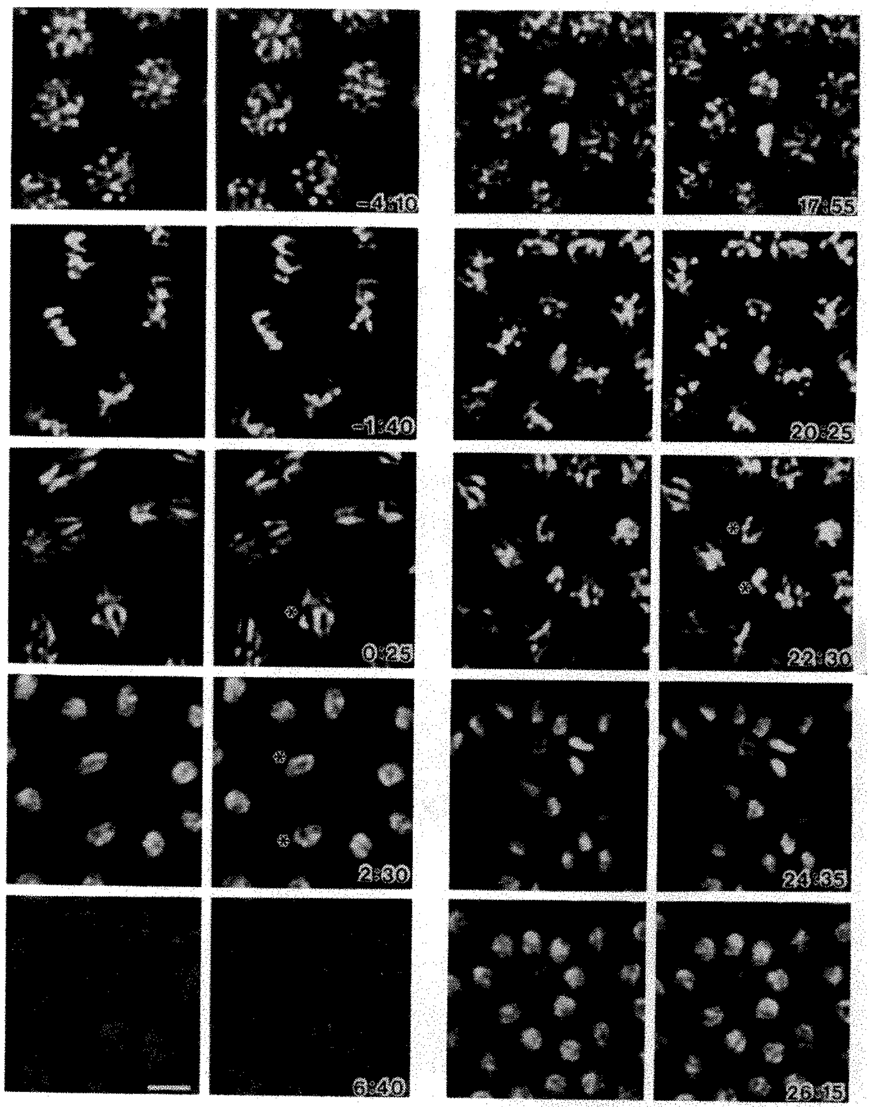

FIG. 1 Stereo pairs selected from the time-lapse data. The numbers represent time in minutes:seconds after the 12th nuclear division. Time is stated relative to the onset of chromosome separation at the metaphase–anaphase transition; a negative sign is given to data dets taken before the 12th nuclear division. Asterisks indicate damaged nuclei (see text and also Fig. 2). Scale bar represents 5 μm.

METHODS. Recording three-dimensional data from living embryos: A standalone, computer workstation CCD system was used which provides real-time capability with a lower digital resolution (12 bits versus 14 bits) but a faster readout rate (200,000 pixel per second versus 50,000 pixel per second) than our previously reported system[5]. All aspects of microscopy, data collection, data storage, image processing and display can be performed on the one workstation. A Peltier-cooled CCD camera (Photometrics, Tucson, Arizona) with a 1340 × 1037 pixel CCD chip (Kodak-Videk) coated to improve short-wavelength response[6], is attached to an IMT-2 Olympus inverted microscope. Microscope shutter, focus movement and CCD data collection are controlled by a MicroVax III workstation coupled to a 20 MFLOPS Mercury Zip 3232 + array processor (Mercury Computer Systems, Lowell, Massachusetts) and a Parallax model 1280 graphic display system having 12 Mbyte of image memory (Parallax Graphics, Santa Clara, California). Calf thymus histones were bound to double-strand DNA cellulose to form nucleosomes, labelled with 5(6)-carboxytetramethyl rhodamine *N*-hydroxysuccinimidyl ester (Molecular Probes, Eugene, Oregon), and eluted with a salt gradient. Rhodamine-labelled histones H2A and H2B, at 0.4 mg ml^{-1}, were microinjected into *D. melanogaster* embryos. Details of the method are reported elsewhere[3]. The rhodamine-histone injected embryos were illuminated with a 100W mercury arc lamp attenuated 20-fold by a neutral density filter and observed using an Olympus 40×/NA1.3 oil immersion objective lens and a high selectivity rhodamine filter combination (Omega Optical, Brattleboro, Vermont). To speed up the image readout, images were taken on a small portion of the CCD, 256 × 256 pixels corresponding to 44 × 44 μm area with a pixel size of 0.17 μm. Five optical section images were recorded at an interval of 1 μm with an exposure time of 0.1 s. It took 5 s to read out correct and store each image; thus, 25 s for one cycle of a focus series. This was repeated 80 times to form a time course. Generation of a time-lapse stereo movie: To make a pair of stereo projections, each set of 5 optical sections was projected to form a single image for each eye. While forming the projection for the left-eye, images were sequentially shifted by 1 pixel to the left, whereas right eye images were shifted 1 pixel to the right. As the optical sections are separated by 1 μm, a shift by one pixel (0.17 μm) per every section (1 μm) corresponds to a tilt of 9.6°, therefore the stereo pair is separated by a tilt of 19.2°. To remove out-of-focus information from the projected images, a three-dimensional optical transfer function was experimentally determined for the objective lens under the conditions used for the data collection of nuclei by imaging very small fluorescent beads (0.1 μm in diameter) as described previously[5]. The projected stereo images were computationally processed to remove out-of-focus information by a constrained iterative deconvolution method using the $Z^* = 0$ plane of the three-dimensional optical transfer function[4]. This approach, based on the Fourier projection theorem, can provide nearly perfect signal recovery using data collection conditions that cause minimum exposure to the specimen. Although not precisely correct for the sliding stereo projections used here, the approximation is suitable for the limited spatial regions examined. For rapid movie display, the entire set of time-lapse stereo images was loaded into the Parallax display system with a 12 Mbyte image memory before the display. This permits a pair of the stereo projection images to be displayed side-by-side and rapidly unpdated to form a time-lapse stereographic movie.

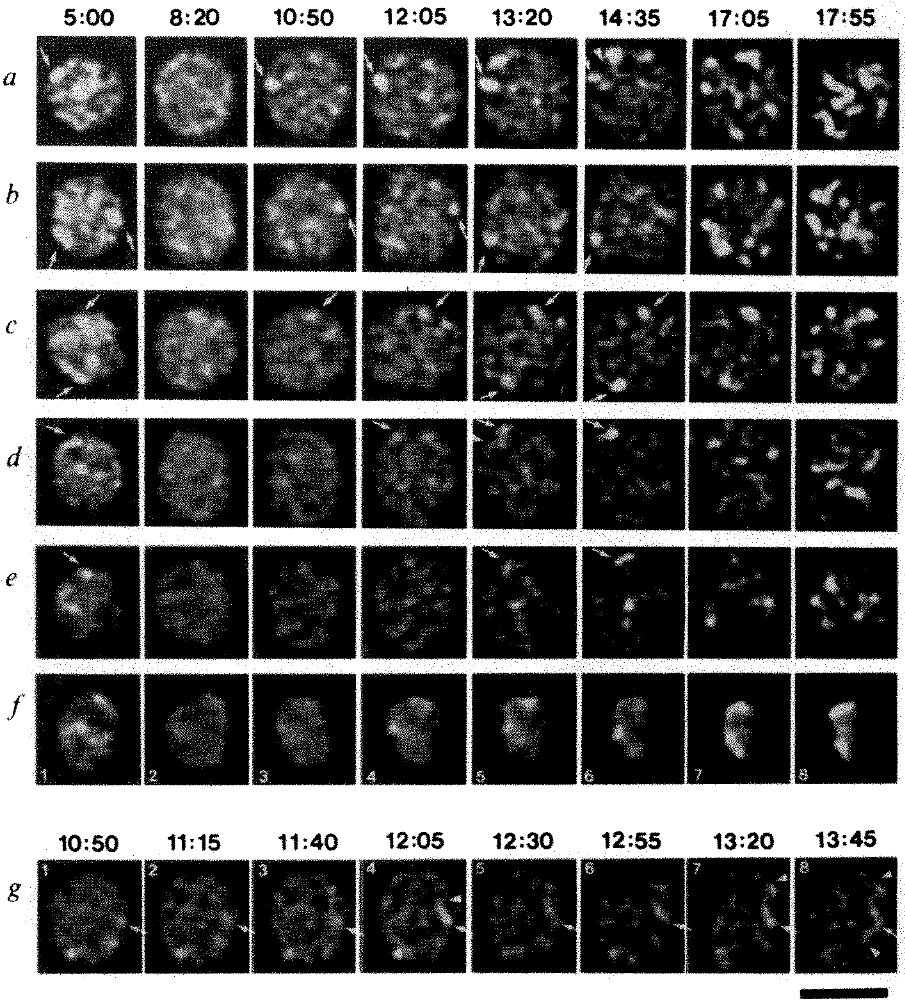

FIG. 2 Chromosome decondensation and condensation. Chromosome decondensation and condensation is shown as a time course. Only one view of the stereo movie is shown for each time point. A damaged nucleus is shown in *f*. Chromosome fibres growing in a single nucleus shown in *g* at every 25 s from 10 min 50 s to 13 min 45 s. The numbers at the top of each column or frame represent time in minutes:seconds as in Fig. 1. Nucleation sites are shown by arrows and fibres by arrowheads. Scale bar represents 5 μm.

appear over a 5-minute interval (Fig. 2*a*–*e*, columns 3–6). Significantly, the relative positions of these early condensation sites were similar to those of the late decondensation sites. Examples of the congruity as shown in Fig. 2*a*–*e* (compare column 1 with columns 3–6). In addition, newly condensed chromosome 'fibres' can be seen elongating from these sites at the rate of about 1 μm per min (Fig. 2*a*, *d* and *g*). In one particularly clear case (Fig. 2*g*), the elongation appears to be bidirectional. As the condensation sites were found near the nuclear midline, they are unlikely to be either centromeric or telomeric sites, which are known to be localized to the apical and basal portions of the nucleus respectively[9]. Towards prophase (Fig. 2*a*–*e*, columns 7, 8), the chromosomes are seen to condense further, forming more contiguous structures. By the time condensation has proceeded sufficiently to identify individual chromosomes, the sites have detached and moved from the nuclear envelope (compare columns 6 and 7 in Fig. 2*a*–*e*), making it impossible to assign these sites, as yet, to specific chromosomes.

Similar chromosomal bright spots were observed in higher-resolution optical section data of nuclear-cycle-13 embryos that had been fixed and stained with the DNA-specific fluorescent dye, 4′,6-diamidino-2-phenylindole (DAPI), as shown in Fig. 3. Below the nuclear midline, chromosome condensation sites were exclusively observed at the nuclear envelope. By contrast, sites were seen both internally and at the periphery above the midline. It seems that the internal bright spots are associated with, and likely to be a part of, the centrometric heterochromatin. Although such peripheral bright spots had been repeatedly observed in fixed preparations in our laboratory, it is only as a result of continuous observation of chromosome behaviour in individual nuclei that we appreciated the significance of these chromosomal regions.

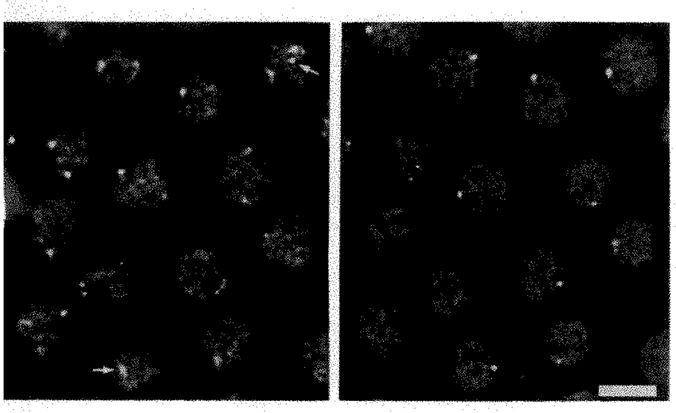

FIG. 3 Sites of condensed chromatin in fixed nuclei. Two optical section images are shown, separated by 2 μm, from a high-resolution three-dimensional data set of a fixed embryo. Because of the curvature of the embryo surface, the focal plane intersects nuclei seen in the upper and lower portions of the field at higher positions than those located in the centre of the field. All of the nuclei shown in the left panel are visualized above the nuclear midline, whereas those in the right panel are all at, or below, the midline. Arrows indicate apparent intranuclear sites above the midline. Scale bar represents 5 μm.

METHODS. *Drosophila* embryos were fixed with 3.7% formaldehyde in heptane/buffer A (15 mM PIPES, pH 7.0, 80 mM KCl, 20 mM NaCl, 0.5 mM EGTA, 2 mM EDTA, 0.5 mM spermidine, 0.2 mM spermine, 0.1% 2-mercaptoethanol). The chorion and vitelline membrane were removed as described previously[17]. The embryos were stained with 0.1 μg ml^{-1} DAPI. Optical section images were collected on the CCD at focus step of 0.2 μm using an Olympus oil-immersion objective lens (60×/NA1.4) and computationally processed to remove the out-of-focus information as described[4].

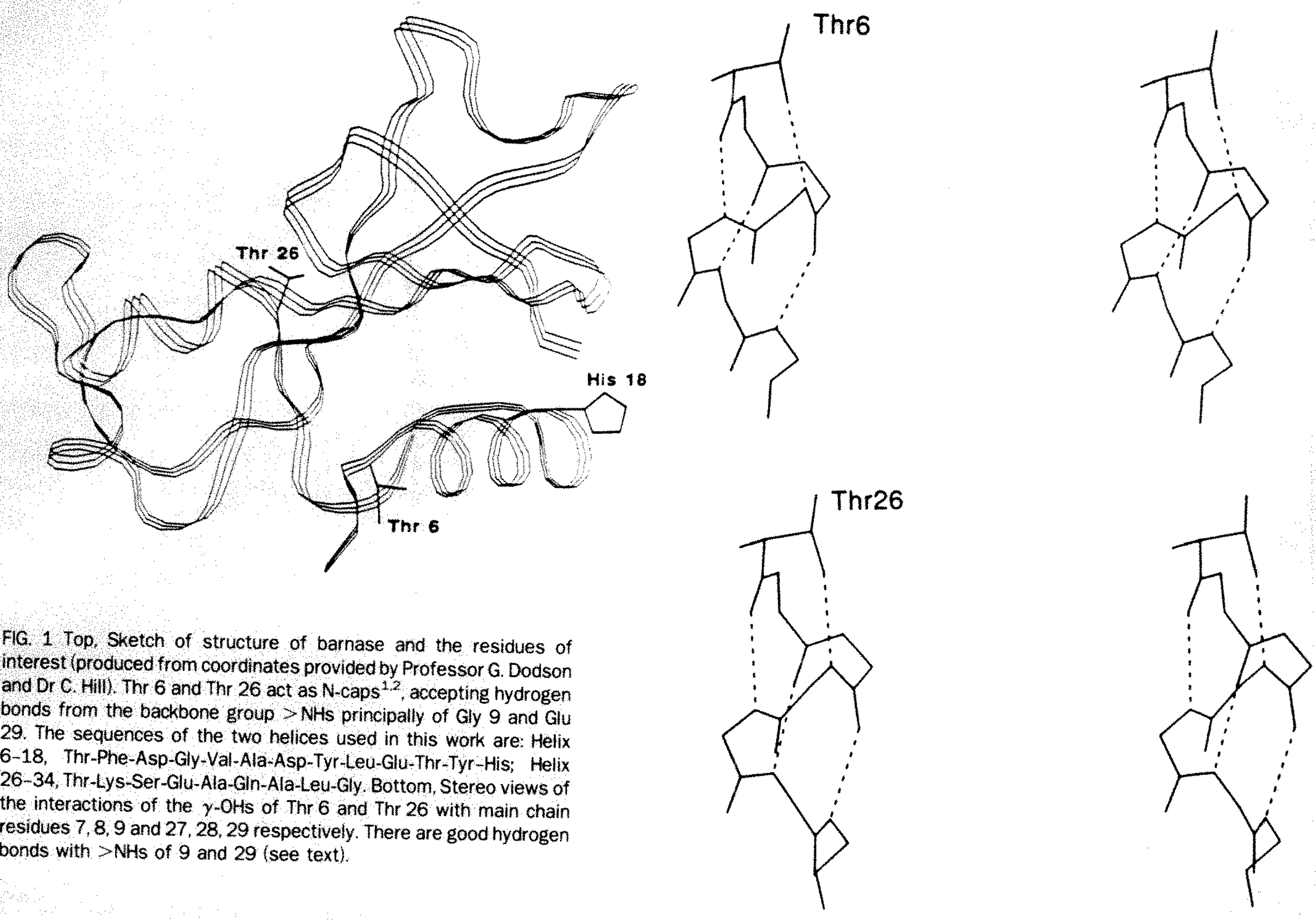

FIG. 1 Top, Sketch of structure of barnase and the residues of interest (produced from coordinates provided by Professor G. Dodson and Dr C. Hill). Thr 6 and Thr 26 act as N-caps[1,2], accepting hydrogen bonds from the backbone group >NHs principally of Gly 9 and Glu 29. The sequences of the two helices used in this work are: Helix 6–18, Thr-Phe-Asp-Gly-Val-Ala-Asp-Tyr-Leu-Glu-Thr-Tyr-His; Helix 26–34, Thr-Lys-Ser-Glu-Ala-Gln-Ala-Leu-Gly. Bottom, Stereo views of the interactions of the γ-OHs of Thr 6 and Thr 26 with main chain residues 7, 8, 9 and 27, 28, 29 respectively. There are good hydrogen bonds with >NHs of 9 and 29 (see text).

wild-type and mutant proteins[5-8]. Barnase contains two α-helices[9], one from residues 6 to 18 and the other from 26 to 34 (Fig. 1). Both α-helices begin with a threonine residue as N-cap (the first residue in the helix[2], namely Thr 6 and Thr 26, Fig. 1), but the overall composition of the rest of each helix is quite different. The -OH groups of Thr 6 and Thr 26 are positioned so that the oxygen atom may act as a hydrogen bond acceptor from the backbone > NH groups of residues 7 or 27, 8 or 28 and 9 or 29 (N1, N2 and N3 in the Richardson[2] nomenclature). The geometry for hydrogen bonding from the -OH of residues 6 and 26 is good with respect to residues 9 and 29, fair for 8 and 28 and poor for 7 to 27 (Table 1), which is usual with N-caps[2]. The C_γ and C_β of both threonines participate in further interactions in the protein (Table 1). Here we use inferences from recent surveys of helix structure[1,2] to design changes in both barnase helices to give precise information on how hydrogen bonding, and electrostatic[10] and other interactions at the N-terminus of a helix affect its stability.

The most common residues found at the N-cap position of helices are: Ser, Asn, Gly, Asp and Thr[2]. The residues Ala, Leu, Val, Ile, Trp, Arg, Gln and Glu are found rarely[2]. We substituted Thr with the following residues: with Gly, Ala and Val to measure the effect of losing hydrogen bonds from the γ-OH to the > NH groups of the helix and to assess the influence of a different size of side chain; with Ser to measure the loss of hydrophobic interactions from the γ-methyl; with Asn and Gln to determine the effects of substituting the carboxyamide oxygen of each of the γ-OH as a hydrogen bond acceptor, and with Asp and Glu to establish the effects of a charged hydrogen bond acceptor. Two mutants, Thr 26 → Asp and Thr 6 → Val, could not be expressed, despite numerous attempts using this expression system[11].

Mutation of either Thr 6 or Thr 26 at the N-caps of each helix in barnase gives quite similar changes in stability and consistent trends (Table 2). Considering that the two helices have different compositions and environments, the results are not specific to a particular helix and so presumably report on some general features. A fine-structure analysis can be made by comparing pairs of mutants that differ by just a small change.

Thr → Ser. Mutation of Thr 6 → Ser causes the loss of 0.22 kcal mol^{-1} and Thr 26 → Ser, 0.56 kcal mol^{-1} of stabilization energy (Table 2). The $C\gamma$ of Thr 6 and 26 make van der Waals' contacts with the protein (Table 1), and so this contribution to the stabilization is lost. It should be noted that none of these contacts is within the helix. Thus, the advantage of having a threonine rather than a serine at the N-cap seems to depend mainly on the existence of tertiary interactions elsewhere in the structure, and not on interactions within the helix itself. These interactions will vary from protein to protein.

Ser → Gly, Ala or Val. Naïve reasoning would suggest that mutation of Ser → Ala might give an empirical measurement of energy change due to the loss of the hydrogen bonds from the -OH to the backbone > NH groups, and Ala → Gly, a measure of the loss of the van der Waals' energy from the β-methyl group. Mutation of Ser 6 → Ala loses 2.3 kcal mol^{-1} or 1.6 kcal mol^{-1} for replacement of Ser 26 (Table 2). But, mutation of Ser → Gly loses only 1 kcal mol^{-1}. Thus, Gly is more stable than Ala at the N-cap by 0.5–1 kcal mol^{-1}. In general, Ala is energetically preferred to Gly in the helix, except at the N-cap[12], either because of the greater entropic freedom of glycine-containing peptides in the denatured state[13], or because of hydrophobic interactions of the alanine β-methyl group in the helix. Why then is Gly preferred to Ala at the N-cap? We suggest that one contributing factor is that the β-methyl group of Ala sterically interferes with solvation of the >NH groups at the N-terminus of the helix—Gly allows more space for optimal hydrogen bonding by water. Consistent with this notion is that Thr 26 → Val is the most destabilizing mutation, despite the retention of the γ-methyl group with its potential for hydrophobic interactions worth ~0.6 kcal mol^{-1}. The large side chain of Val makes solvation of the >NH groups by water difficult. These results parallel statistical data on the frequency of residues at the N-cap (Table 3). There is evidence elsewhere that an uncharged hydrogen bond contributes some 0.5-1.5 kcal mol of stabilization energy to a complex[14]. The apparent binding energy of the N-cap hydrogen bond from Ser to the >NH groups is in this region.

Thr → Asn, Gln. The previous mutations are relatively easy to interpret in the absence of detailed crystal structures of the mutants because interactions were deleted without introducing new ones, and so the necessary structural information is present in the crystal structure of the wild type. Substitution with Asn, Asp (or Glu or Gln), adds the possibility of further interactions that can obscure simple interpretation. Statistically, Asn is one of the most favourable residues at the N-cap of helices[2]. The carbonyl oxygen of the side chain can act as a hydrogen bond acceptor of the backbone >NH groups. On the other hand, Gln and Glu are rarely found at the N-cap as their side chains cannot make good hydrogen bonds to the backbone >NH groups[2]. Mutation of Thr → Gln in positions 6 to 26 results in destabilizing the protein by 1.9 and 1.7 kcal mol^{-1} respectively. But Thr → Asn also destabilizes both helices by 1.3 kcal mol^{-1}. The reason for this is not obvious, because Asn can be built into the structure with its carbonyl oxygen placed to accept hydrogen bonds from the backbone >NH groups and its own $-NH_2$ group exposed to water. Perhaps Asn is not inherently as good an N-cap as Ser or Thr, but may be better when the $-NH_2$ group can hydrogen bond elsewhere in the protein. There should be more protein structure surveys undertaken to look for other factors. Whatever the explanation, Asn is not necessarily a good choice for the N-cap of an helix.

Thr → Asp, Glu. Mutation of Thr 6 → Asp stabilizes the protein by 0.1 kcal mol^{-1}, but Thr 6 → Glu destabilizes it by only 0.3 kcal mol^{-1} and Thr 26 → Glu destabilizes it by less than 0.1 kcal mol^{-1}, despite the low frequency of Glu at the N-cap[2]. The α-helix possesses a macroscopic dipole with its positive pole at the N-terminus[10]. Recent studies have suggested values of about 1.4-2.1 kcal mol^{-1} for the interaction energy of a single charge with the dipole of a helix[6,15]. A charge-dipole electrostatic interaction is the probable reason for the stability of the mutants with Asp and Glu. Comparison of Asp with Asn and Glu with Gln gives another estimate of the importance of the charge-dipole interaction: Asp 6 → Asn gives 1.4 kcal mol^{-1}; Glu 6 → Gln, 1.6 kcal mol^{-1}; and Glu 26 → Gln, 1.6 kcal mol^{-1}. These results are strikingly similar to those of the earlier studies, despite

TABLE 1 Contact distances (< 5 Å) from Thr 6 and Thr 26 to other residues*

Atom	Neighbouring atom	Distance (Å)
Thr 6		
Oγ	N (Phe 7)	3.63, 3.53, 3.80†
	N (Asp 8)	4.17, 4.06, 4.00†
	N(Gly 9)	3.57, 3.14, 3.05†
Cγ	O_{δ} (Asn 5)	3.83
	C (Asn 5)	3.81
	O (Asn 5)	4.20
	O_{δ} (Asn 77)	3.98
C_{β}	N (Phe 7)	3.05
	N (Asp 8)	4.55
	N (Gly 9)	4.73
	C (Asn 5)	3.64
	O (Asn 5)	4.15
Thr 26		
Oγ	N (Lys 27)	3.37, 3.34, 3.67†
	N (Ser 28)	3.54, 3.38, 3.77†
	N (Glu 29)	3.35, 2.94, 3.23†
	$O_{\delta 1}$ (Asp 54)	4.20
	$O_{\sigma 2}$ (Asp 54)	4.77
Cγ	C (Ile 25)	4.04
	O (Gly 52)	3.52
	C (Gly 53)	4.48
	O (Gly 53)	4.47
	$O_{\delta 1}$ (Asp 54)	4.07
	$O_{\delta 2}$ (Asp 54)	4.66
Cβ	N (Lys 27)	3.08
	N (Ser 28)	4.30
	N (Glu 29)	4.66
	O (Ile 25)	4.34
	C (Ile 25)	3.70
	O (Gly 52)	4.17
	Cγ (Asp 54)	3.88
	$O_{\delta 2}$ (Asp 54)	3.74

* Contact distances from Thr 6 and Thr 26 to other residues were obtained from X-ray coordinates (2 Å resolution), kindly provided by Professor G. Dodson and Dr C. Hill.

† There are three molecules in the unit cell, and these are the individual values for each molecule. The accuracy for bond distances at this resolution is about ±0.2 Å.

TABLE 2 Change in free energy of unfolding ($\Delta\Delta G_U$) on mutation of N-caps (Thr 6 and Thr 26)*

	$\Delta\Delta G_U$ (kcal mol^{-1})	
Mutation	Position 6	Position 26
Thr → Ser	0.22	0.56
Thr → Val		2.31
Thr → Ala	2.53	2.11
Thr → Gly	1.34	1.58
Thr → Asn	1.27	1.29
Thr → Asp	−0.11	
Thr → Gln	1.87	1.72
Thr → Glu	0.27	0.05
Asp → Asn	1.38	
Glu → Gln	1.60	1.67
Asp → Glu	0.38	
Asn → Gln	0.60	0.43
Ser → Ala	2.31	1.55
Ser → Gly	1.12	1.02
Gly → Ala	1.19	0.53

* $\Delta\Delta G_U$ is the free energy of unfolding of wild-type enzyme minus that of mutant. The free energies of unfolding of wild-type protein and mutants were analysed by urea denaturation monitored by fluorescence[5-7]. This analysis is based on the equation $\Delta G_U = \Delta G_U^{H_2O} - m[\text{Urea}]$, where ΔG_U is the free energy of unfolding in urea solution, $\Delta G_U^{H_2O}$ the free energy of unfolding in water, and m is a constant. We find that there is little difference in calculating $\Delta\Delta G_U$ directly by comparing ΔG_U for wild type and mutant at the same concentration of urea[5] or by using the simpler procedure[7] of $\Delta\Delta G_U = \langle m\rangle(\Delta[U]_{1/2})$, where $\Delta[U]_{1/2}$ is the concentration of urea at which wild type is 50% denatured, minus the concentration of urea at which the mutant is 50% denatured. The average value of m for wild type and mutant is denoted by $\langle m\rangle$. We find that m does not deviate by more than 10% of a mean value which is 2.23 (±0.02 standard error) kcal mol^{-2} for all mutants. $[U]_{1/2}$ never differs by more than 1% (< ±0.04 M) for experiments performed over a period of time and is generally reproducible to ±0.01 M with identical batches of urea. The data are probably reliable to ±0.02 kcal mol^{-1}. As the free energy of unfolding determined by either method is essentially the same, we have used the second method method to obtain the values of the free energy of unfolding of all the proteins.

Proteins were prepared by an improved procedure. Wild-type enzyme was secreted from *E. coli* BL21DE3 pLysS (ref. 16) harbouring the barnase gene construction[12] on the pTZ18u plasmid (Pharmacia). Cells were grown in low-phospate medium[17] and the expression of barnase was induced by the addition of 0.5 mM isopropyl-β-D-thiogalactoside following the conditions described by Studier *et al.*[16]. After further growth for 2.5 h, the protein was purified as before[18]. For site-directed mutagenesis we used the method of Eckstein *et al.*[19]. Mutants were identified either by direct sequencing of single-stranded DNA or after previous screening by the hybridization method[20].

TABLE 3 Comparison of statistical and energetic surveys of N-cap stabilities

Preferred residue at N-cap	Stabilization energy relative to Thr (kcal mol^{-1})*	Frequency at N-cap†	Relative value‡
Asp	−0.1	27	2.1
Thr	0.0	21	1.6
Glu	0.2	5	0.4
Ser	0.4	34	2.3
Asn	1.3	34	3.5
Gly	1.5	33	1.8
Gln	1.8	3	0.4
Ala	2.3	10	0.5
Val	2.3	1	0.1

* Average value at positions 6 and 26.

† Frequency found in survey in ref. 2.

‡ Frequency at N-cap normalized for frequency of occurrence in proteins in general[2].

there being an unfavourable electrostatic interaction at position 6 with the negative charge on Asp 8. As so many different substitutions with different hydrogen bonding patterns and at different ends of the helix give similar values, the estimate of 1.4–2.1 kcal mol^{-1} for the helix–charge interaction is valid.

There is a very rough parallel between the energetics of N-cap formation and statistical surveys of the frequencies found in practice (Table 3). Notable exceptions, however, are Asn and Glu. Our data indicate that Glu is quite acceptable at the N-cap, being only marginally less stable than Thr. Asn is statistically over-represented, given the data in this study.

In conclusion, the N-cap is important to stability and substitution of selected residues can cause an energy change of over 2 kcal mol^{-1}. It is clear, however, that when designing a helix, the choice of residue depends on the context of the overall tertiary structure—that is, on interactions with residues removed in sequence but stereochemically close to the helix. Even so, it seems that some residues are particularly favourable. For example, Ser is inferior to Thr only when hydrophobic interactions are favourable for the γ-methyl group. For barnase, the Ser mutants are only 0.2 to 0.6 kcal mol^{-1} less stable, although the methyl group can be worth up to 1.5 kcal mol^{-1} when packing is particularly good[5,7]. In other circumstances, steric exclusion of the γ-methyl group would prohibit the use of Thr. Asp is clearly the best for an exposed N-terminus that has no adjacent negative charges. ☐

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1. Presta, L. G. & Rose, G. D. *Science* **240,** 1632–1641 (1988).
2. Richardson, J. S. & Richardson, D. C. *Science* **240,** 1648–1652 (1988).
3. Kim, P. S. & Baldwin, R. L. *Nature* **307,** 329–334 (1984).
4. Shoemaker, K., Kim, P. S., York, E. J., Stewart, J. M. & Baldwin, R. L. *Proc. natn. Acad. Sci. U.S.A.* **82,** 2349–2353 (1985); *Nature* **326,** 563–567 (1987).
5. Kellis, J. T. Jr, Nyberg, K., Sali, D. & Fersht, A. R. *Nature* **333,** 784–786 (1988).
6. Sali, D., Bycroft, M. & Fersht, A. R. *Nature* **335,** 741–743 (1988).
7. Kellis, J. T. Jr, Nyberg, K. & Fersht, A. R. *Biochemistry* **28,** 4914–4922 (1989).
8. Matouschek, A., Kellis, J. T., Serrano, L. & Fersht, A. R. *Nature* **340,** 122–126 (1989).
9. Mauguen, Y. *et al. Nature* **297,** 162–164 (1982).
10. Hol, W. G. J. *Prog. Biophys. molec. Biol.* **45,** 149–195 (1985).
11. Paddon, C. J. & Hartley, R. W. *Gene* **53,** 11–19 (1987).
12. Strehlow, G. H. & Baldwin, L. R. *Biochemistry* **28,** 2130–2133 (1989).
13. Matthews, B. W., Nicholson, H. & Becktel, W. J. *Proc. natn. Acad. Sci. U.S.A.* **84,** 6663–6667 (1987).
14. Fersht, A. R. *Trends Biochem. Sci.* **12,** 301–304 (1987).
15. Nicholson, H., Becktel, W. J. & Matthews, B. W. *Nature* **336,** 651–656 (1988).
16. Studier, F. W., Rosenberg, A. H. & Dunn, J. J. *Meth. Enzym.* (in the press).
17. Serpesu, E. H., Shortle, D. & Mildvan, A. S. *Biochemistry* **25,** 68–87 (1986).
18. Mossakowska, D. N., Nyberg, K. & Fersht, A. R. *Biochemistry* **28,** 3843–3850 (1989).
19. Sayers, J. R. & Eckstein, F. in *Genetic Engineering: Principles and Methods* Vol. 10 (ed. Setlow, J. K.) 109 (Plenum, New York and London, 1988).
20. Carter, P. J., Winter, G., Wilkinson, A. J. & Fersht, A. R. *Cell* **38,** 835–840 (1984).

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X-ray analysis of HIV-1 proteinase at 2.7 Å resolution confirms structural homology among retroviral enzymes

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KNOWLEDGE of the tertiary structure of the proteinase from human immunodeficiency virus HIV-1 is important to the design of inhibitors that might possess antiviral activity and thus be useful in the treatment of AIDS[1]. The conserved Asp–Thr/Ser–Gly sequence in retroviral proteinases[2] suggests that they exist as dimers similar to the ancestor proposed for the pepsins[3–5]. Although this has been confirmed by X-ray analyses of Rous sarcoma virus and HIV-1 proteinases[6,7], these structures have overall folds that are similar to each other only where they are also similar to the pepsins[8]. We now report a further X-ray analysis of a recombinant HIV-1 proteinase at 2.7 Å resolution. The polypeptide chain adopts a fold in which the N- and C-terminal strands are organized together in a four-stranded β-sheet. A helix precedes the single C-terminal strand, as in the Rous sarcoma virus proteinase[6] and also in a synthetic HIV-1 proteinase, in which the cysteines have been replaced by α-aminobutyric acid[9]. The structure reported here provides an explanation for the amino acid invariance amongst retroviral proteinases, but differs from that reported earlier[7] in some residues that are candidates for substrate interactions at P_3, and in the mode of intramolecular cleavage during processing of the polyprotein.

To obtain HIV-1 proteinase for crystallization, a recombinant fragment of the viral *pol* gene was expressed in *E. coli* as described in the legend to Fig. 1. Crystals, prepared using the published protocol[10], grew at 4 °C in five days. X-ray data were collected from a tetragonal bipyramidal crystal to 2.7 Å resolution using a FAST area detector. Earlier, we constructed models using Composer[5], initially from the pepsins and later from the Rous sarcoma virus (RSV) structure. From these we constructed a model for use in molecular replacement that did not include the N- or C-terminal strands, the helix or the flap, the positions of which were uncertain. A series of difference Fourier analyses, modelling, and refinement steps gave first the region 47 to 54 (the flap), then the N- and C-termini, and finally the turn after the N-terminal strand (residues 5 to 11) and the α-helix (residues 87 to 93). In each case the polypeptide could be followed in the difference electron density (calculated on the basis of a model in which they had not previously been included) without a break and with clear positions for the side chains (Fig. 1). Thus we achieved an unequivocal tracing of all 99 residues of the polypeptide chain. Further details of the analysis and refinement are given in the legend to Fig. 1. The final geometry has a 0·019 Å r.m.s. deviation from idealized geometry and the present agreement value is 18.9%.

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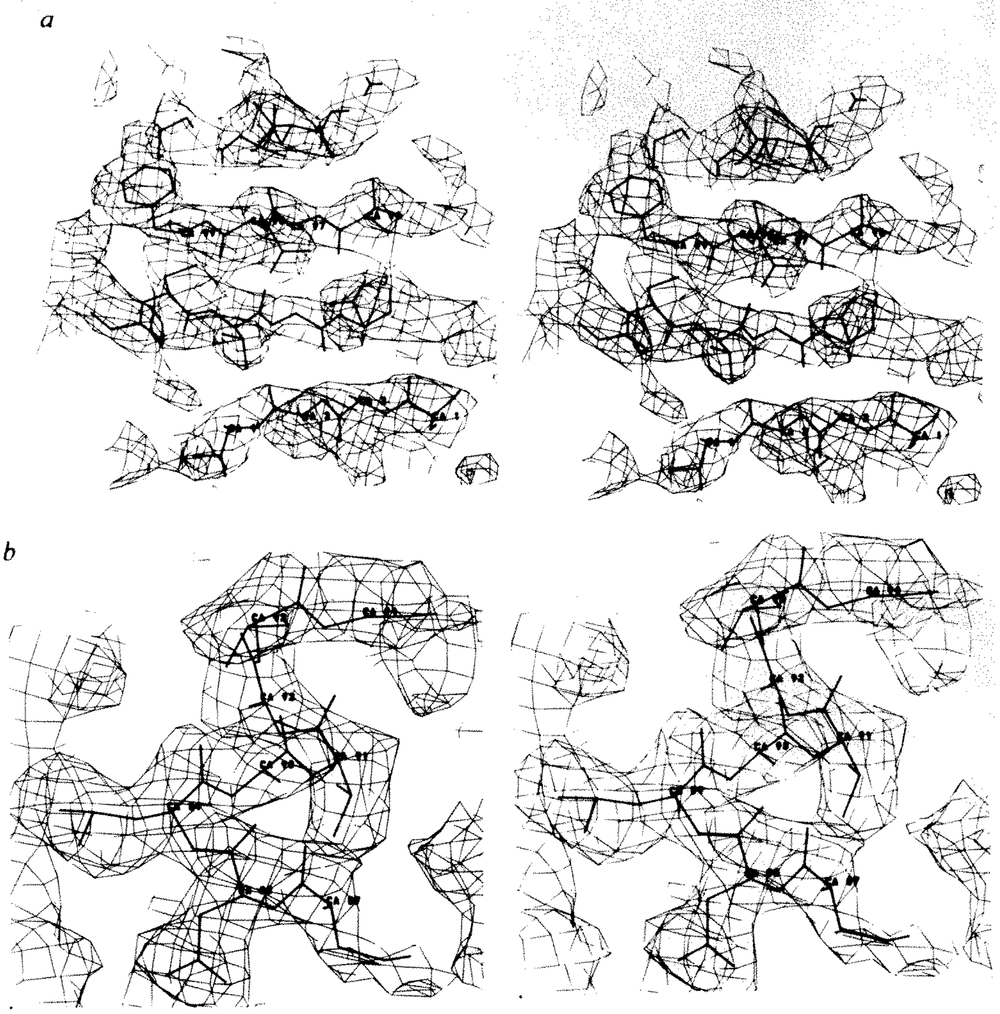
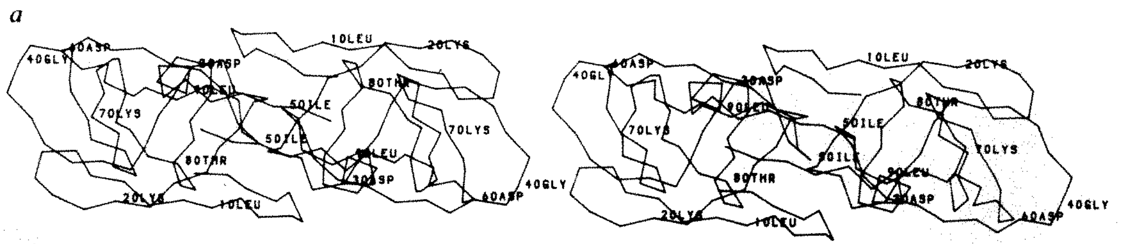


FIG. 1 Stereo views of difference electron density maps calculated with coefficients $2F_{obs} - F_{calc}$ for *a*, the intermolecular four-stranded, antiparallel β-pleated sheet involving the N-terminal and C-terminal strands (a and t) and *b*, residues 87 to 90 which form part of the helix, h′, preceding the terminal strand, t; the strands and helices here are defined in the legend to Fig. 2 and were omitted from the calculation of phases used in the difference Fouriers.

METHODS. Enzyme for crystallization was expressed in *E. coli* as follows. The bacterial expression plasmid pHIVexpol5 was constructed by subcloning the 5.0 kilobase *Bgl*II fragment from the pHXBc2 clone of the viral genome[18] into the *Bgl*II-cleaved derivative of pPFZ-R2 (ref. 19). This gave a gene controlled by the *trp* promoter and encoding a fusion polypeptide comprising an initiator methionine, a 7-residue junction sequence Ala-Glu-Ile-Thr-Arg-Ile-Glu-, and a fragment of the *pol* gene product which had a relative molecular mass of 115,000 (115K) which encompassed the proteinase, reverse transcriptase and integrase domains. *E. coli* MM294 cells harbouring pHIVexpol5 were shown by western blotting and immunodetection to produce appropriately processed proteinase (11K). HIV-1 proteinase was purified from the soluble fraction of cell lysates (D.E.D. *et al.*, manuscript in preparation) to give a single band on Coomassie blue-stained SDS-polyacrylamide gels. Automated Edman degradation gave an N-terminal sequence of Pro-Gln-Ile-Thr-Leu-, consistent with the expected result of self-processing of the *pol* gene product. The purified proteinase recovered per litre of bacterial culture was 0.25 mg. The specific activity of the purified enzyme was measured using a 17-residue synthetic peptide substrate (RRVTNSATIMMQRGNFR (one-letter code); J. Schneider, personal communication), and was estimated at 2.4 μmol substrate cleaved per min per mg of proteinase at pH 5.5, 23 °C and 0.2 mM substrate. Details of the expression studies, cell growth and protein purification will be presented elsewhere (D.E.D. *et al.*, manuscript in preparation). Crystals, isomorphous with those reported earlier[7] of space group $P4_12_12$, were used for FAST area detector data collection, giving 10,000 reflections over a period of two days. A merging *R*-value was 0.11 leading to over 2,200 reflections with $I > 2.0\sigma(I)$, with a maximum resolution of 2.7 Å. Refinement of the initial model using rigid group refinement was achieved using RESTRAIN[20] using the CRAY XMP/28 at University of London Computing Centre. In the final cycles, we used individual thermal parameters but tight geometric restraints. Difference Fouriers calculated with coefficients $2F_{obs} - F_{calc}$ and $F_{obs} - F_{calc}$ were displayed simultaneously using FRODO[21] on an Evans and Sutherland PS390. The model was extended in steps to include the N- and C-termini and the helix and at each stage refined using restrained least squares.



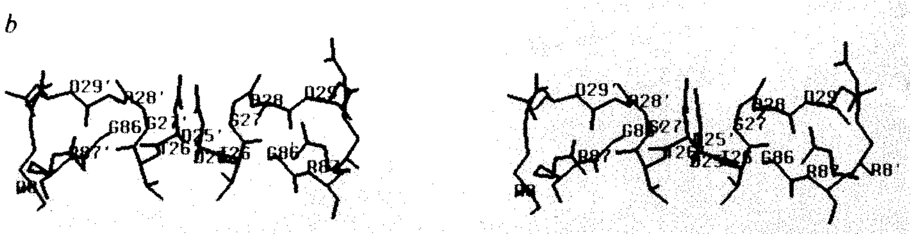


FIG. 2 Stereo views of *a*, C_α atoms viewed from the side of the dimer containing the four-stranded β-sheet and along the twofold axis relating subunits in the dimer, and *b*, conserved residues of the active-site cleft (Asp 25, Thr 26, Gly 27, Asp 29 and Arg 87) which are organized around the twofold axis of the dimer and are thought to be involved in substrate recognition and catalysis.

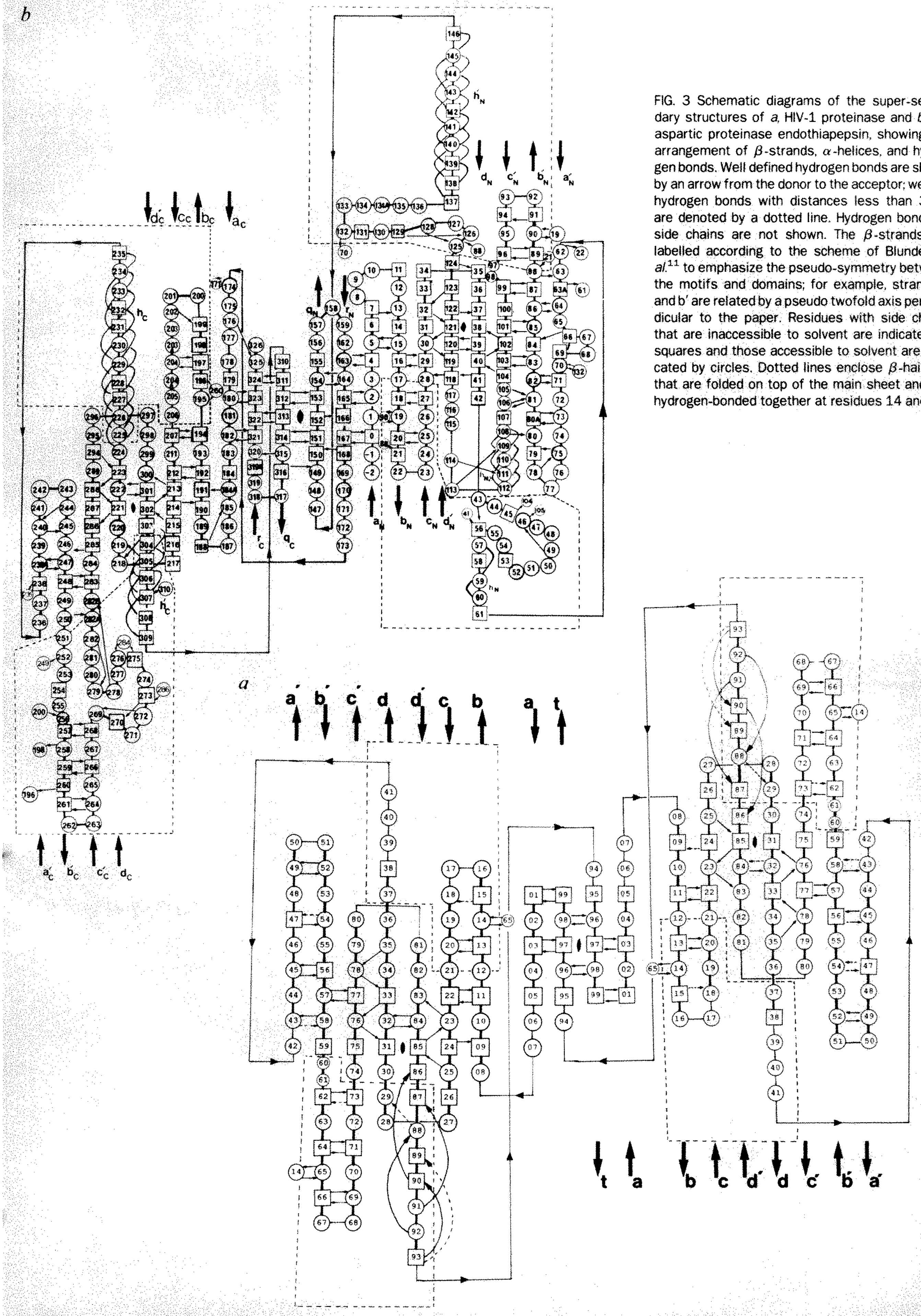


FIG. 3 Schematic diagrams of the super-secondary structures of *a*, HIV-1 proteinase and *b*, the aspartic proteinase endothiapepsin, showing the arrangement of β-strands, α-helices, and hydrogen bonds. Well defined hydrogen bonds are shown by an arrow from the donor to the acceptor; weaker hydrogen bonds with distances less than 3.9 Å are denoted by a dotted line. Hydrogen bonds to side chains are not shown. The β-strands are labelled according to the scheme of Blundell *et al.*[11] to emphasize the pseudo-symmetry between the motifs and domains; for example, strands b and b′ are related by a pseudo twofold axis perpendicular to the paper. Residues with side chains that are inaccessible to solvent are indicated by squares and those accessible to solvent are indicated by circles. Dotted lines enclose β-hairpins that are folded on top of the main sheet and are hydrogen-bonded together at residues 14 and 65.

Figure 2 shows stereo views of the three-dimensional structure of the enzyme, which exists as a dimer with a well defined and extensive active-site cleft. Figure 3 shows the secondary structure and hydrogen bonding in the dimeric structure compared with that of a pepsin. We adopt the strand nomenclature[11] suggested for endothiapepsin. Each subunit comprises two similar motifs related by an approximate twofold axis; each motif contains anti-parallel strands: a, b, c and d for the first and a', b', c' and d' for the second. These are organized together in a distorted sheet, with strands c and d' and strands c' and d forming two pairs of parallel strands (Fig. 3). Strands b and c and strands b' and c' form antiparallel β-hairpins which are folded on top of the first sheet and hydrogen-bonded together around the same twofold axis that relates the motifs in the main sheet. The strands d and d' are followed by an irregular region and an α-helix, respectively, which also lie on the same side of the main mixed β-sheet. Strand a of the first motif is displaced from the main mixed sheet and forms an antiparallel four-stranded β-sheet with the terminal strand, t, and the two equivalent strands from the other subunit of the dimer. These strands occupy the same volume but have different orientations from the six-stranded β-pleated sheet that connects the two domains of the pepsins (Fig. 3*b*).

The structure corresponds closely to the arrangement of the completely symmetrical dimer that has been proposed as the ancestor of the aspartic proteinases[3]. The conserved active site residues, Asp 25–Thr 26–Gly 27, form a symmetrical and highly hydrogen-bonded arrangement virtually identical to that described for pepsins[12,13]. The two threonines are inaccessible to solvent and are hydrogen-bonded so that the O-gamma bonds to main-chain N–H and C=O functions of the other subunit in a fireman's grip. The two aspartates lie roughly coplanar, with their inner carboxylate oxygens hydrogen-bonded to the N–H functions of Gly 27. There is density for a water molecule bound equally to the two carboxylates, as found in pepsins.

An extensive hydrophobic core involving residues indicated by squares in Fig. 3 extends through the dimeric interface. Pro 1, Ile 3 and Leu 5 of the N-terminal strand, and Cys 95, Leu 97 and Phe 99 of the C-terminal strand, form one side of the central four-stranded β-pleated sheet and pack onto Leu 24 and Thr 26 adjacent to the catalytic residues and the hydrophobic residues of the helix, such as Leu 89, Leu 90 and Ile 93. These residues of the core tend to be highly conserved amongst the retroviral proteinases, giving support to this interpretation of the structure. The only truly buried polar residue apart from Thr 26 is Thr 31, which is also hydrogen-bonded to main-chain N–H and C=O functions in a fashion that is often important to protein structure. Thr 31 is either retained as threonine or occasionally conservatively varied to serine in retroviral proteinases.

One of the significant conservations in the consensus sequences of the retroviral and pepsin-like aspartic proteinases is an amino-acid sequence of two hydrophobic residues followed by a glycine, an example of which is the sequence Ile 84–Ile 85–Gly 86 that is found in HIV-1 proteinase. The retroviral proteinases differ, however, in the presence of an invariant arginine in the next position, for example Arg 87. Such a basic residue is never found in the pepsins. The crystal structure of HIV-1 proteinase shows that this conserved arginine participates in an intersubunit cluster of ionic and hydrogen bonding interactions involving the side chains of the conservatively varied Asp 29 and Arg 8, as in RSV proteinase[6]. No reason for the conservation of these residues is apparent from the structure reported by Navia *et al.*[7]. It is also apparent that the N-terminus is well organized in the dimer and so flexibility cannot be involved in the precursor autocleavage[7].

The close similarity of the three-dimensional structures of the dimeric retroviral proteinases and the monomeric pepsins suggests a common mode of interaction with substrate. X-ray studies of aspartic proteinase transition-state isostere complexes[14–16] show that the substrate probably binds pseudo-symmetrically in the active-site cleft, using topologically equivalent hydrogen bonding functions and specificity pockets on each side of the scissile bond. By analogy, the carbonyls of Gly 27 in HIV-1 proteinase may form hydrogen bonds to the substrate N–H functions of P_1 and P_2', as suggested by Blundell and Pearl[8] and by Wlodawer and coworkers[17]. The conservatively varied but exposed Leu 23, Ala 28, Val 82 and Ile 84 form the large S_1/S_1' specificity subsites. By analogy with the aspartic proteinases, in which P_3 binds through its main chain to the side-chain oxygen and the main-chain N–H of Thr 219 (pepsin numbering), these interactions would be mediated by Asp 29 in HIV-1 proteinase. This gives further support to the importance of the cluster of charged residues involving Asp 29; the charged residue Arg 87 contributes to the correct orientation of the carboxylate group (Fig. 2*b*). The pocket, S_3, in this HIV-1 proteinase structure differs from that reported earlier[7] in that it involves the loop between strands a and b.

The perfectly symmetrical structure of the retroviral proteinases lends credence to the suggestion[13] that the apparently differing pK_as of the two aspartates in aspartic proteinases are more a function of their proximity than of their differing environments. Nevertheless, the symmetrical dimer must lose its perfect symmetry during catalysis as the substrate has only an approximate dyad. But this is not a restriction for inhibitors, so symmetrical structures may have a major advantage in high affinity binding when compared with an analogous substrate. ☐

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1. Kohl, N. E. *et al. Proc. natn. Acad. Sci. U.S.A.* **85,** 4686–4690 (1988).
2. Toh, H., Ono, M., Saigo, K. & Miyata, T. *Nature* **315,** 691 (1985).
3. Tang, J., James, M. N. G., Hau, I.-N., Jenkins, J. A. & Blundell, T. L. *Nature* **271,** 618–621 (1978).
4. Pearl, L. H. & Taylor, W. R. *Nature* **329,** 351–354 (1987).
5. Blundell, T. L. *et al. Eur. J. Biochem.* **172,** 513–520 (1988).
6. Miller, M., Jaskolski, M., Rao, J. K. M., Leis, J. & Wlodawer, A. *Nature* **337,** 576–579 (1989).
7. Navia, M. A. *et al. Nature* **337,** 615–620 (1989).
8. Blundell, T. L. & Pearl, L. H. *Nature* **337,** 596–597 (1989).
9. Wlodawer, A. *et al. Science* **245,** 616–621 (1989).
10. McKeever, B. M. *et al. J. biol. Chem.* **264,** 1919–1921 (1989).
11. Blundell, T. L., Jenkins, J. A., Pearl, L.-H., Sewell, T. & Pedersen, V. in *Aspartic Proteinases and Their Inhibitors* (ed. Kostka, V.) 151–161 (de Gruyter, Berlin, 1985).
12. James, M. N. G. & Sielecki, A. *J. molec. Biol.* **163,** 299–361 (1983).
13. Pearl, L. H. & Blundell, T. L. *FEBS Lett.* **174,** 96–101 (1984).
14. Bott, R., Subramanian, E. & Davies, D. R. *Biochemistry* **21,** 6956–6962 (1982).
15. James, M. N. G., Sielecki, A. R., Salituro, F., Rich, D. H. & Hofmann, T. *Proc. natn. Acad. Sci. U.S.A.* **79,** 6137–6142 (1982).
16. Blundell, T. L. *et al. Biochemistry* **26,** 5585–5590 (1987).
17. Weber, I. T. *et al. Science* **243,** 928–931 (1989).
18. Ratner, L. *et al. Nature* **313,** 277–284 (1985).
19. Franke, A. E. Eur. Patent 0147178 (1985).
20. Haneef, I., Moss, D. S., Stanford, M. J. & Borkakoti, N. *Acta crystallogr.* **A41,** 426–433 (1985).
21. Jones, T. A. *J. appl. Crystallogr.* **11,** 268–272 (1978).

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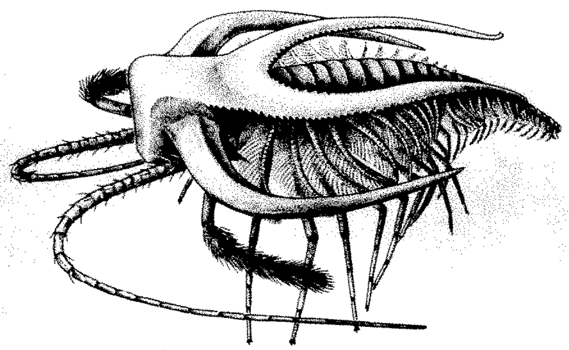
Richard A. Fortey

Wonderful Life: The Burgess Shale and the Nature of History. By Stephen Jay Gould. *W. W. Norton: 1989. Pp. 346. $19.95. To be published in Britain by Hutchinson, February 1990.*

THE Burgess Shale, which crops out high in the Rockies of western Canada, is a deposit of mid-Cambrian age. It was discovered in the last century by the great American palaeontologist C.D. Walcott, and preserves an astonishing array of soft-bodied animals which allows us a glimpse of the true range of organisms present at an early stage in metazoan history. In *Wonderful Life* Stephen Jay Gould describes the investigation of this famous fossil fauna: "the world's most important fossil animals".

Gould brings alive the monographs and descriptions of these animals which H.B. Whittington and his colleagues have prepared with such care over the past two decades. He presents the unravelling of the morphology of the remarkable finds as an intense psycho-drama, slapping on a bit of hyperbole here, and a colourful analogy there, to buoy the reader through passages that might seem too technical in any other hands. There is no question about the historical importance of the Burgess Shale, and Gould is right when he says that it deserves a place in the public consciousness along with big bangs and black holes.

But the story is directed to a particular end, and it is this end which, to Gould, lends the Burgess fauna its real interest. His contention is that here we see "a disparity in design far exceeding the modern range throughout the world" (p.62). Organisms from the Burgess Shale may belong to phyla unknown in the living fauna; the arthropods — the most important element in the fauna — do not conform to modern groups, with few but important exceptions. Instead we have a time of wild experiment, with morphological components picked like items from a menu in a Chinese restaurant, and placed together in combinations that no longer exist. The subsequent, post-Cambrian, history of life is seen as one of loss of designs — impoverishment, if you like — in spite of the wondrous variety of the modern world. This is surely a dramatic story, but is it true?

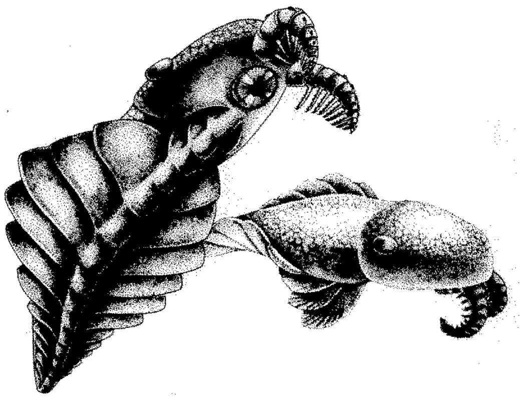


Marella — small and elegant.

Gould follows a traditional line in taxonomic attitude. The taxonomic status (equivalent to importance) of a group is decided by what he terms 'disparity', which means the morphological 'distance' from other groups. A breakthrough occurred, he maintains, when the Burgess arthropods were recognized as including "a series of unique designs, beyond the range of later groups" (p.168). The most peculiar animals of all might be designated as hitherto unknown phyla, the highest taxonomic division below the Kingdom. Curiously, Gould hardly mentions the new discipline of cladistics, which was being developed at exactly the same time as Conway Morris, Briggs and Whittington were patiently chipping out Burgess oddities. The cladistic method works by analysing shared similarities, and produces a classification based on derived, homologous characters; and it does not make value judgements about 'disparity'.

The problem with 'disparity' is that its estimation depends on the authority of the expert: how is he to know what makes — what is 'worth' — a phylum? Or what a class? For the Burgess Shale the answer seems to be lack of success in assigning the taxon into a known, living group, so that the fossil becomes 'an animal with no modern relatives'. But unless the animal arose afresh from the mud it *has* to have some modern relatives, be they ever so distant. The end product of the 'disparity' approach seems to be the insouciant recognition of a new phylum, as if it were only to be expected (on p.143 Gould quotes one prominent researcher as remarking, "Oh fuck, another new phylum", on the discovery of a new form).

For systematic affinities to be discovered, the arthropods from the Burgess Shale *can* and should be analysed in terms of the characters they share rather than those they uniquely possess. They seem to fall into a rather ordered array, with *Marrella*, Walcott's original 'lace crab', as the most primitive member, and the trilobites surprisingly advanced. A similar exercise on echinoderms has seen the positioning of numerous so-called Cambrian 'classes' within a coherent phylogenetic picture. The advantage of this kind of analysis is that it suggests further fruitful questions. For example, the arthropods are metameric organisms, and the characteristic arrangements of appendages (legs, gills and the like) of groups living today may have been assembled piecemeal; the Burgess Shale animals, for all their oddity, may record the stages in this assembly. Hence the fossils may be vital to the understanding of arthropod phylogeny; their apparent 'disparity' may partly reflect the process of assembly of advanced clades.



Anomalocaris — a "capable swimmer".

Scientific discussion will centre on the homology of particular characters, which is always controversial in arthropods. But sorting out such complex questions provides more constructive insights into the classification of these arthropods than simply saying: "Gee isn't that weird — it must be a new class!". One might suspect that if, say, the stalked goose barnacles had been known from the Burgess Shale alone ("an arthropod-like animal covered in calcareous plates and with a flexible stalk — goodness gracious!") they would have found themselves in a new phylum. Yet we know from their development that they are specialized crustaceans. It is about time that organisms were classified by characters they share rather than by intuitions about 'disparity'.

Whatever one makes of their relationships, Gould is surely correct in saying that these animals are among the most important for understanding the history of life. Study of them has demanded patience and persistence, and has mostly been carried out with microscope and pencil and paper rather than the high-technology, high-expenditure approach typical of fashionable science. *Wonderful Life* is a compelling story, told with characteristic verve. □

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Drawings, by Marianne Collins, are taken from the book reviewed here.

erotic pictures).

Meantime, Sherpa Stafford is slaving away at hush-hush experiments to prove Cantor's cancer theory. These are so hush-hush and *so* exhausting that they drive Celestine to complain about his sexual inadequacies, as she soaps his nether regions, using that four-letter word which Ken Tynan — like Carl Djerassi — did not shrink to use. Eventually, Stafford succeeds and all is set for the vital letter to *Nature* which eventually yields the "Nobel in medicine". But not before a lot of worry and unpleasantness about the inability of Kurt Krauss, I.C.'s nasty competitor at Harvard Medical School, to repeat poor Stafford's work. Had Jerry fiddled the results? We are not really sure even when Celestine is disturbed with another boyfriend — appropriately called Roger — to learn that Jerry is to share the Nobel prize with I.C.

Celestine goes along to Stockholm with her Auntie Paula and they all have a *wonderful* time — pages of it — with both I.C. and Jerry spouting T.S. Eliot in their speeches and Jerry having a nice gossip with the Swedish Queen about how the Brits use their knives and forks and eat peas.

And that's about it really, apart from some reasonably happy endings — all neatly tied up — and a final bitter letter to I.C. from poor old Krauss, who is still as sick as a parrot about not getting his Nobel prize.

What, I wonder, were Professor Djerassi's motives in writing this book? Is he condoning what he portrays? Is he saying, look, this is how it is. What a smart lot we scientists are: pushy, clever, cutting corners (nudge, nudge), getting a bit on the side (snigger, snigger), swanking about our erotic pictures and Boccherini! If he is, then the hell with it. If, on the other hand, he is saying, look, this rat race is awful, then *Cantor's Dilemma* is a clever, if stomach-turning, way of portraying it. □

John Treherne, novelist and scientist, died on 23 September. This rumbustious review was characteristic of the writer and the man.

Ideal thinking

Steven Rose

Russian Psychology: A Critical History. By David Joravsky. *Basil Blackwell: 1989. Pp. 583. £45, $34.95.*

IMAGINE a book entitled *American Psychology: A Critical History,* the first third of which advances that history no further than 1917, the bulk of which follows events only to the early 1930s, and which devotes a mere 30 pages to the years since 1953 – years that have seen such a flowering of the neural and behavioural sciences, and the transformation of our understanding of brain and behaviour. That this disproportion does not seem wholly incongruous for a Western history of Soviet psychology is of course indicative of the extraordinary interest that the first 36 years of the tortured (and torturing) relationship of Soviet communism with science has held for political philosophers, historians and scientists alike. Nor would a history of American psychology of the period (unless of course written by Mr Leo Abse, MP, the recent biographer of Britain's present Prime Minister) be likely to find space for a psychological analysis of F.D. Roosevelt, as this book offers for Stalin. But it also reveals the limits of that interest, for Stalin's death in 1953 is precisely the midway point between October 1917 and the current era, and the second half of this period should surely be as relevant and subject to historical scrutiny as the first for what claims to be, as Joravsky's book does, a comprehensive account.

The superficial view of Soviet science under Stalin sees the period straightforwardly as one in which scientific data and theory were subordinated to political ideology, typified by the Lysenko episode in genetics, often regarded as a simple reciprocal of Nazi eugenics. Such an account misses the passionate concern of Marxism to see itself simultaneously as science and metascience, and the equally powerful determination of an impoverished and backward country to embrace and use science in its desperate push towards modernization. Equally, an unquestioning acceptance of the Western liberal view of science as neutral and above politics, philosophy or ideology misses the opportunity to see, in that Soviet experience, a mirror of some of the problems about the place and organization of science in our own society.

A number of Western scholars have tried to move beyond this simplistic view; the Lysenko affair has been reassessed by Levins and Lewontin, by Lecourt and by Joravsky himself, while Loren Graham's comprehensive *Science and Philosophy in the Soviet Union* has recently been revised and republished. Joravsky's earlier essay in this direction, *Soviet Marxism and Natural Science,* was for a long period after its appearance in 1961 the only book on the theme in English.

The development of Soviet psychology is of particular interest. First, psychology had strong roots in pre-revolutionary Russia, stretching back through Pavlov to Sechenov. Second, the subject matter of psychology has profound bearing on theories of human nature and society, and hence *a fortiori* on Marxism. Third, the theoretical battle between materialism and dualism, between reductionism and dialectics, which in many areas of science is masked or confined to the philosophers, has been and remains a vital issue of methodology and scientific practice for research in psychology and neurobiology in the West as well as the East. Finally, the vast experiment in social engineering in the Soviet Union during the first half of its post-revolutionary history was directed to the creation of "the new socialist man [*sic*]", and one might well have expected that psychologists would find themselves mobilized to this task.

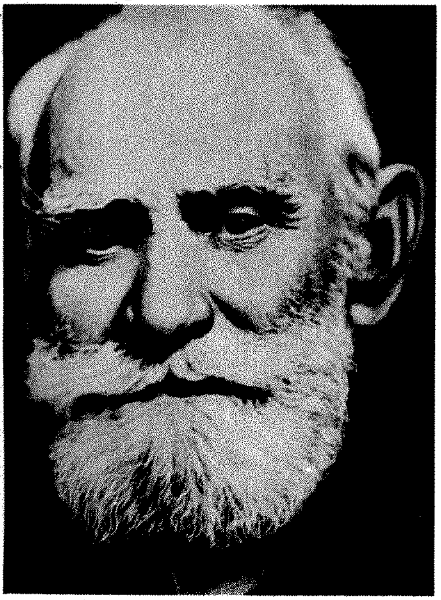
Thus, from the 1920s on, Soviet neurophysiologists, psychologists and philosophers struggled to develop an adequate theory of the dialectic of brain and society in the production of mind, and an adequate practice of pedology (educational science) and psychotechnics based upon that theory. This struggle with some of the deepest conceptual and methodological problems with which science has to cope, produced responses ranging from the hard-line physiological materialism of Pavlov almost until his last days, through the pioneering work on the social production of mind by the polymath Vygotsky and the later development of neuropsychology by Luria. In the next generation it produced Rubinshtein's activity theory, Anokhin's functional systems approach, the work of Beritashvili, Leontiev and Orbeli, as well as a strong school of functional neurochemistry.

And of course, the work of nearly all of these scientists was punctuated by endless crises, from the desperate economic situation and famine of the early years (to be repeated during the 'Great Patriotic War'), through the increasing tendency to solve scientific disputes by administrative fiat – with all the dread consequences that that entailed – from the 1930s to well beyond the death of Stalin. The debates in psychology seem not to have been quite so literally life-and-death affairs as they were in genetics, although Joravsky lists many victims of the Gulag. True, after 1945 Pavlov was for a time elevated to stardom within Stalin's pantheon, and recantation was forced upon those of his pupils and disciples whose greater understanding of brain physiology and psychology (to say nothing of their better Marxism) led them to deviate from the line of their late master. Even so, the debates in the 'Pavlov sessions' for psychologists, physiologists and psychiatrists in 1950, 1951 and 1952 seem much less staged and more open than the notorious 1948 session of the Agricultural Academy which saw the destruction of genetics and the apotheosis of Lysenko.

Just why this should be so would seem to be a question of some significance – perhaps it was because of the stronger research and philosophical traditions of Soviet psychology and physiology;

perhaps because the declared luminary of the subject, Pavlov, unlike Lysenko, was long dead; perhaps because there was a greater urgency to solve the practical crisis in agriculture than to employ science instead of naked coercion in the production of the new socialist man.

Oddly, however, although Joravsky documents the debates extensively he makes no great effort to address these broader questions, and the omission is characteristic of a book which in the last analysis yields much less than it promises. Joravsky is not an internalist historian; he is not so much concerned with experimental findings which led Pavlov, Kornilov, Vygotsky or Luria to their theoretical positions, or with the ebb and flow of the scientific debate; rather, he is offering us a metahistory of psychology, an account of the ways in which the leading protagonists of the discipline in the 1920s and 1930s sought to locate their theories within the swirling currents of the party-led attempts to establish a Marxist orthodoxy. In effect this book is not so much a history of Russian psychology but of psychology in Russia.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Pavlov — developing theories.

The author's scholarship is formidable, and the phrenology he has created whilst denied entry into the Soviet Union or access to many key papers is impressive, despite the fact that the book has, on internal evidence, been a very long time in the making and is the weaker for it. Nonetheless, Joravsky creates the strong impression of being in the last analysis rather bored by the scientific questions which dominate the professional lives of the subjects of his history. A reader of this book who opened it knowing nothing even of Pavlov's key experiments and theories would close it being little the wiser, though he would have learned that Pavlov was a vain, opinionated and authoritarian little man whom Joravsky doesn't like very much. Indeed, Joravsky finds it difficult to observe admirable qualities in almost any of his protagonists. If they don't speculate about the broader significance of their results he finds them narrow technocrats; if they do, they are shallow philosophers, or time-servers who would mould their theories to political exigencies. The treatment — or rather dismissal — of Rubinshtein, Luria and Anokhin in this way is inexcusable in however critical a history. A couple of what Joravsky would probably regard as boringly internalist histories of Russian psychology were published some years ago and do a much more useful job in this regard.

Joravsky would probably respond by saying he wasn't trying to write that sort of book at all. His elegantly worked-over prose, full of ironic asides, musing self-reflections and studied ambiguities may in some ways best be read as an unspoken historiographic debate with what has become known as the Edinburgh school in the sociology, history and philosophy of science, which seeks to reduce debates about fact and theory in science to a reflection of the interests — the social origins and positions — of the main characters. Joravsky's own interest, as the doyen of the history of Soviet science in the West, remains somewhat unstated. He offers himself as an outsider, neither a sympathizer with nor enemy to communism, an olympian observer and chronicler. But in practice he cannot sustain such detachment; his language constantly lets him down, as when, for all his reading of the Marxist classics, confronted with that robust concept 'working class' he routinely bowdlerizes it to 'lower class'. A real critical history of Soviet psychology, which transcends internalist–externalist boundaries, and helps us to rescue its rational kernel from both Eastern and Western ideological trappings, remains to be written. It will be worth waiting for. ☐

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Pens and petals

Ray Desmond

The Art of Botanical Illustration: The Classic Illustrators and Their Achievements from 1550 to 1900. By Lys de Bray. *Christopher Helm, London: 1989. Pp. 192. £25.*

The drawing of flowers — whether for research, decoration or simply for pleasure — is the subject of an impressive literature. A distinguished pioneer in this field was Agnes Arber, whose survey of printed herbals and their woodcut illustrations, *Herbals: Their Origin and Evolution* (1912; 2nd edn, 1986), has become a classic. Another well-known and authoritative text is Wilfrid Blunt's *The Art of Botanical Illustration* (1950; 3rd edn, 1955). His *Illustrated Herbal* (1980), written in conjunction with Sandra Raphael, complements Arber's volume in dealing primarily with the manuscript herbal.

These are just three of many books dealing with aspects of botanical art that have appeared during the course of this century, many of them during the 1970s and 1980s. Artists such as Leonardo de Vinci, Albrecht Dürer, Jacques le Moyne, Georg Ehret, Sydney Parkinson, Pierre-Joseph Redouté, Mrs Delaney and Marianne North have all been the subject of monographs. Other authors have looked at flower drawing and painting from the point of view of the bibliographer and the printer, while exhibitions, museums and auction houses have each generated accounts of specific collections.

Any new book in this area has to be judged against the high standards set by some of its predecessors. Even though Lys de Bray's *The Art of Botanical Illustration* has no pretensions to original research, aimed as it is at a general readership, and is generously illustrated with colour plates, it is an unhappy piece of work. There are frequent signs of haste in its preparation and the text bristles with errors — misspellings, mistakes in dates of birth and death and in dates of publication — some of which are doubtless due to careless proof reading.

These slips are irritating but others are more misleading. Jacobus van Huysum was employed by Sir Robert (not Horace) Walpole; William Kilburn did not contribute any plates to the *Botanical Magazine*; B. Maund and E.D. Smith were connected with the *Botanic Garden* not the *Botanical Register*; J.N. Fitch lithographed plates for the *Botanical Magazine* but he did not succeed his uncle as artist to the journal. There is no evidence that William Kent landscaped the Prince of Wales's garden at Kew (which was much more than the 11 acres stated by Ms de Bray); Princess Augusta did not extend her property at Kew after the death of Queen Caroline; and W.T. Aiton did not resign from Kew in 1841, he merely relinquished control of the small botanical garden there. This is not an exhaustive list.

It is unfortunate that the author should have chosen *The Art of Botanical Illustration* as her title because it invites comparison with Wilfrid ('Wilfred' in Ms de Bray's book) Blunt's volume. Clearly, though, it poses no threat to the status of the earlier work. ☐

Ray Desmond is at the Royal Botanic Gardens, Kew, Richmond, Surrey TW9 3AB, UK.

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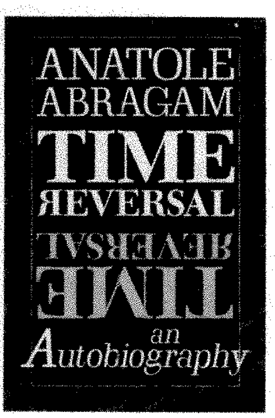

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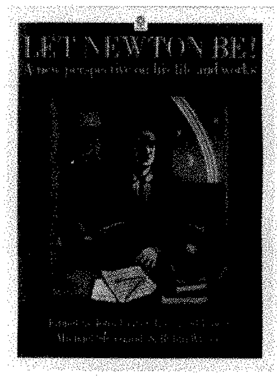

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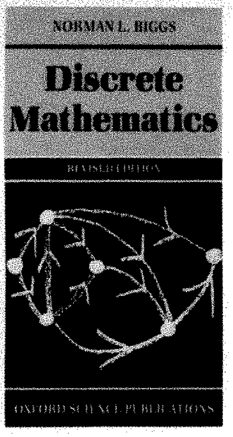

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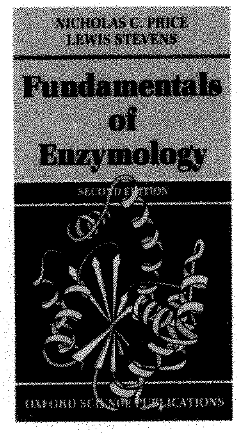

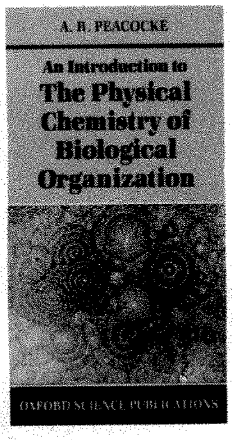
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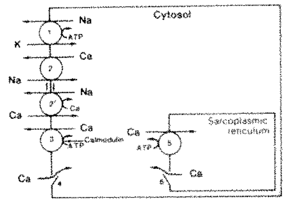

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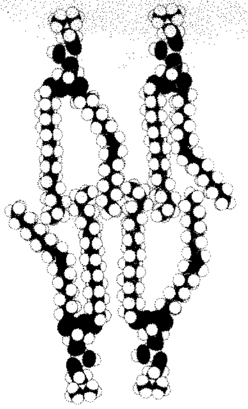

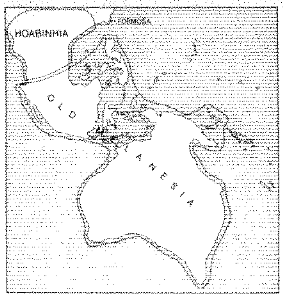

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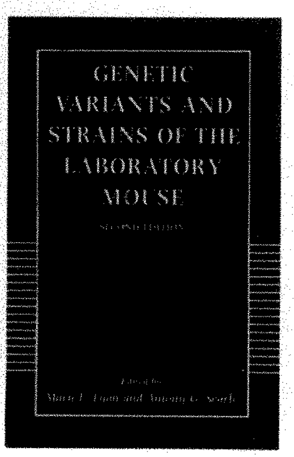

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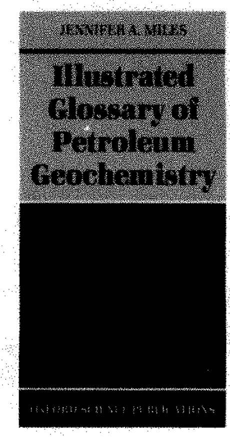

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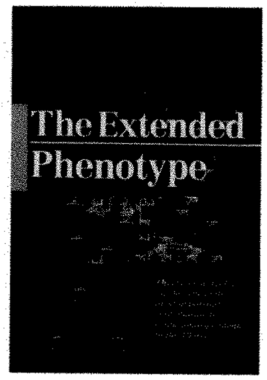

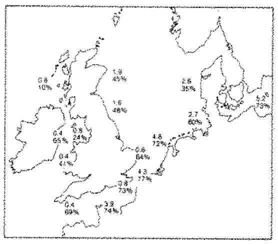

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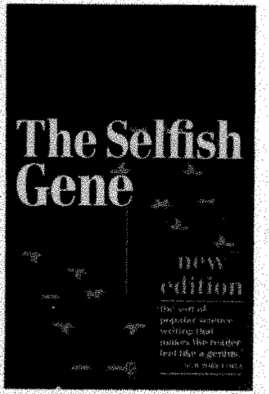

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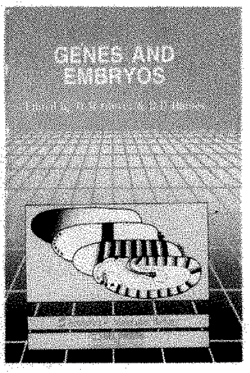

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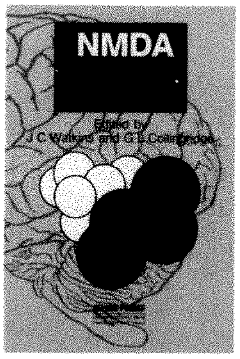

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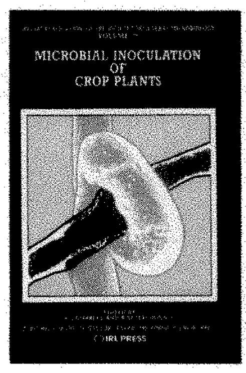

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Adventures of Ben Trovato

Walter Gratzer

Discovering: Inventing and Solving Problems at the Frontiers of Scientific Knowledge. By Robert Root-Bernstein. *Harvard University Press: 1989. Pp. 501. $35. To be published in Britain next month, £27.95.*
Fortune or Failure: Missed Opportunities and Chance Discoveries in Science. By Alexander Kohn. *Basil Blackwell: 1989. Pp. 199. £14.95, $24.95.*
Serendipity: Accidental Discoveries in Science. By Royston M. Roberts. *Wiley: 1989. Pp. 270. Hbk $19.95, £13.30; pbk $12.95, £8.65.*
Harrap's Book of Scientific Anecdotes. By Adrian Berry. *Harrap, London: 1989. Pp. 240. £12.95.*

MURPHY's law, or as the French call it, *le loi d'emmerdement maximum,* dictates that when we drop the breakfast toast on the newspaper or floor it always falls spread-side down. Imagine now recovering the errant morsel and finding, when you turn it over to scrape away the bits of carpet, the long-lost contact lens embedded in the marmalade. This, according to Royston Roberts, is serendipity. Consider next the case of the American professor of statistics, who observed that when his children dropped their bread and butter on the carpet it always landed buttered-side up. A violation of Murphy's law? By no means, for on looking into the matter more closely the professor found that his children were buttering their bread on both sides. This Dr Roberts calls pseudo-serendipity — an unsought discovery that comes to reward the deserving, those whose minds are equipped to receive and interpret the signal, who descry that tide in the affairs of men, which taken at the flood leads on to fortune.

Should you feel impelled to buy these four books, you will learn, not once but four times, that serendipity is a coinage of Hugh Walpole's, derived from the Persian fable of *The Three Princes of Serendip* (that is to say Ceylon, or if you prefer, Sri Lanka), meaning the happy faculty of making unexpected and fortunate discoveries. Robert Root-Bernstein explores the phenomenon in the greatest depth and his is by far the most ambitious work. It is cast in the form of a running seminar, for six voices, about the nature of the scientific process. One of these, referred to as Imp, I take to be the author's *alter ego.* The discussion ranges over the practice, philosophy, organization and sociology of science with no little erudition, even if one occasionally fancies that one is being beaten about the cranium with it ("As Paul Valéry says . . ."). Imp and his friends develop many a provocative but abundantly documented thesis. Science, they find, for example, is getting worse: ever more scientists are generating less and less new knowledge per head; the success of science at the national level does not reflect the munificence of its funding. Many small countries — Denmark is one — do better than the United States, let alone the Soviet Union, by such criteria as Nobel prizes per head of population.

Root-Bernstein's protagonists argue a great deal about creativity. How may it be promoted and can it be taught? The uneasy outcome seems to be that it can be inculcated by educational precept, according to such formulae as used to be advertised by Edward de Bono; the existence of the great scientific dynasties — Nobel laureates begetting Nobel laureates — suggests, on the other hand, that it is better learned by example, as part of the patrimony of a great laboratory.

Contemporary mistrust of Jenner's theory.

Mary Evans Picture Library

Root-Bernstein illustrates his argument with many examples. He favours research by bold and hazardous flights of the intellect: to be audaciously wrong is better than to be timidly correct. ("This theory isn't right", Debye was supposed once to have declared after a seminar, "it isn't even wrong".) Imp is ever keen to challenge the wisdom of the day. Crick's Central Dogma is his favourite target. But here the urbane mask suddenly slips, revealing, as it seems, a bloodshot glint of messianic fire; for it is Root-Bernstein's own work (published in the *Journal of Theoretical Biology*) that his surrogate cites in evidence. He is out to black the Establishment's eye by breaching the infamous Central Dogma with a demonstration that information can flow from protein to protein. Model building leads him to a scheme of complementary amino acids. In search apparently of a spot of serendipity, he meanders down a byway and arrives at a model for an interaction between a neurotransmitter, serotonin, and myelin basic protein. Imp speaks highly of Root-Bernstein's work. He then goes on to denounce the intolerance of the guardians of entrenched opinion towards such enterprising conceits as four-stranded DNA structures, and the purblind reluctance of grant committees to shell out money for the pursuit of heterodoxy. I find this slightly queasy reading: it seems to me to come close to the cry of the crackpot down the ages, demanding a blank cheque to support what Irving Langmuir called (with criteria by which it could be recognized) pathological science.

Root-Bernstein quotes with approval Albert Szent-Györgyi's remark about the fatuity of a system of funding that required of him some indication of what he proposed to do with the money. If, he maintained, he knew what he was going to discover, there would be no need for him to discover it. Judged by this argument of course all discoveries are by their nature serendipitous. But Root-Bernstein goes on to make a compelling case that the grant system is becoming increasingly rigid, and acts to discourage those digressions in pursuit of the unexpected that have so often led to the most striking discoveries. His analysis of conceptual upheavals in science over the past century or so also leads him to the conclusion that a large proportion have come from unknown researchers, operating alone in the geographical wilderness. If he is right then the cultivation of 'centres of excellence' and the projected elimination of research from minor seats of learning in Britain are a dangerous folly. Our scientific mandarins should examine his evidence with care.

Another of the contentions ventilated by Imp and his companions is that the creative process is nourished by artistic imagination, and that the scientific elect most often possess accomplishments in music, in painting or in poetry. The evidence is marshalled in a table that spreads itself over several pages. This may be right, but for my part I have too often found the giants of our profession to be boorish thugs to be wholly convinced. Moreover, while there may be no doubt about Einstein's love of music, his proficiency on the fiddle by all accounts did not far outstrip that of Heifetz in cosmology. (His frequent partner, Martinu, who wrote a sonatina for him, was supposed to have snapped in exasperation: "Damn it, Einstein, can't you count?")

But enough grousing, for Root-Bernstein has written an absorbing, stimulating, sometimes infuriating book, full of entertaining ideas, and everyone with an interest in science and its ways will benefit from reading it. ▶

Alexander Kohn and Royston Roberts plough the selfsame furrow. All the old tales are here, nor do they fade with the telling, and there are also a good many I did not know. Both miss the story of Dr Murphy's patient in Boston, who was fond of raw liver and became cured of pernicious anaemia, but not much else that I could think of. Kohn concentrates more on medical discovery, and in today's world of mandatory LD50 tests, clinical trials and FDA approval, the casual manner in which dire procedures were tried on human subjects — the family, often enough, of the experimenter — taxes credulity. Jenner, it may be remembered, "with great fortitude tried the cow-pox on his wife and children", but before that he had taken the precaution of inoculating a farm-boy, James Phipps, and then injecting him with the real article — a smallpox extract. Much more recently a research director at Boehringer, screening a series of compounds for vasoconstrictor activity to use against the common cold, tried one out on his snuffling secretary; she promptly sank into a sleep that lasted 24 hours, and her blood-pressure was found to be alarmingly low. By this means clonidine, the sovereign treatment for hypertension, was discovered.

In Memoriam

For "quaker", pray, and for "moth-eaten", mourn.
This threnody recalls their fate forlorn.

We catered to their reproductive need.
We cherished and encouraged them to breed.
We nourished them (for 15 cents a day)
Until, on orders, they were shipped away:
Their natures chromosomally imbued
With genes for "non-immunity" or "nude",
From whites to pure C-57 blacks:
A thousand, thousand cultivated JAX.

In two score years and two, that realm of mice,
Felled once by fire, has now been stricken twice,
Ignited by explosion of propane
To holocaust in far Bar Harbor, Maine,
Where, cage on cage, in box on burning box,
There perished scores of rare foundation stocks.
On shelf and bench, in closely serried racks,
There died a pyre of martyred murine JAX.

Weep, then, for mice in Roscoe Jackson's name,
Incinerated in a burst of flame.
Like fair Brunhild', by fiery circle girt,
They died, the noblest breeds of Mount Desert.
Their bodies ashed, their genes distilled away—

Mourn for "moth-eaten", and for "quaker", pray!

Ralph A. Lewin

In the drug field, especially, the exercise of reason seems to have played little part in the best discoveries. Lithium therapy is an example to make any rational pharmacologist put by his smoked drum and seek a tranquil retirement. Kohn tells the story well: an Australian psychiatrist conceived the notion that manic disease was caused by a toxin, and that this would appear in the urine. So he injected the urine of his patients into guinea pigs. The guinea pigs reacted badly, but less so, he judged, to control urine. The next stop was to identify the toxic principle. Urea killed the guinea pigs, but its concentration was the same in manic and normal urine. How about uric acid then? But free uric acid is insoluble, so the dauntless experimenter prepared the lithium salt. Not only did it appear innocuous, but it counteracted the toxicity of urea and exerted a 'calming effect' on the agitated rodents. To do the psychiatrist justice, he did then ask himself whether it was the uric acid or the lithium that was exerting the benign affect, and indeed lithium carbonate proved equally soothing. He now tried lithium carbonate on a patient, with miraculous results. A paper was written and ignored until it was discovered in the fringe literature by a Danish worker some years later. Lithium therapy became in due time one of the great triumphs of clinical science; but manic patients have no toxin in their urine and the guinea pigs became quiet only because the lithium made them sick.

Roberts is an organic chemist and his trade is perhaps the richest of all in fortuitous and perverse discoveries. He has written an entertaining book, accessible to all. Here is Dr Sapper, who breaks a thermometer in the reaction vessel and is rewarded by the discovery that mercuric ion is a catalyst for the oxidation of naphthalene to phthalic anhydride, and so begets the modern dyestuff industry. Roy Plunket, in search of a better refrigerant, sets out to try a prep with tetrafluoroethylene. The weight of the gas cylinder shows it to be full, but nothing comes out. On being sawn open it yields up a waxy solid, now known as Teflon. A negligent graduate student leaves his cigarette smouldering on the bench while he works; he takes a drag and finds that it tastes intensely sweet. He has discovered saccharine. Forty years later James Schlatter at Searle, pursuing ulcer drugs, is synthesizing a terminal dipeptide of gastrin. He licks his fingers and experiences a sensation of sweetness. He tastes his dipeptide ("It was a bold man who first swallowed an oyster", said King James), and lo, aspartame or Nutrasweet is the fat man's friend. And so it goes.

Who are these holy innocents, whose curls fortune ruffles with her fingers, while giving the rest of us the flat of the hand across the windpipe with the other arm? Roberts believes they are just like us, only quicker of wit and sharper of perception. For every one of them there must be a thousand others, who failed to take the tide at the flood, and the voyage of whose lives was accordingly bound in shallows and in miseries.

Kohn enlarges on the subject of missed opportunities. These tales are inevitably harder to come by, but he tells for example of a well-known biochemist, still among us, who went to the wrong seminar and failed to initiate a collaboration which would probably have started recombinant DNA a year or two early. He might have mentioned also the Japanese physicist, who shelved an observation that should have led him to the discovery of the neutron; it was said that in his later years cruel students would drop the word in his presence to see the tears well up and course down his cheeks. But what of all the discoveries and inventions that have not been made? Like children unconceived, they will never be missed, but one can imagine them massing in the shadows to mock the bureaucrats who work so hard at keeping scientists away from the paths of dalliance.

On his deathbed, Lord Camrose was supposed to have addressed the following exhortation to his assembled family: "If you want to die as rich as I am, never brighten up *The Daily Telegraph*". But do not let this put you off *Harrap's Book of Scientific Anecdotes,* which comes from the pen of the *Telegraph*'s science correspondent. The book is not what its title suggests, but rather a collection of Adrian Berry's favourite passages of scientific and technological journalism, yarns of discovery and exploration from *The New Atlantis* to Chernobyl. It is agreeably written, spiced with witticisms and altogether good fun.

I shall leave you with just one example of Berry's historical research. It concerns the reception given to Alexander Graham Bell's telephone. The mayor of an American city grasped its potential at once: "One day", he said, "there'll be one in every city". But what did the experts think? Here is the Chief Engineer of the British Post Office, reporting with considered wisdom to a House of Commons committee: "Americans have need of the telephone — but we do not. We have plenty of messenger boys". So much for peer review. □

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Sunk without a trace

June Goodfield

The Mind Has No Sex? Women in the Origins of Modern Science. By Londa Schiebinger. *Harvard University Press: 1989. Pp.355. $29.50, £23.50.*

"LET us now praise famous men, and our fathers that begat us", runs the verse from Ecclesiasticus. Not, you notice, that women or mothers were in any way involved nor were they likely to have been present in the writer's mind even as he concluded: "And some there be which have no memorial. Who are buried as though they had never been". It was ever thus, I concluded, as I finished Londa Schiebinger's remarkable book.

Its title comes from an assertion made over 300 years ago by François Pollain de la Barre, as part of an attempt to secure a place for women in the then emerging modern science. The author of this book adds a question mark to his phrase — not because she has any doubts about the matter but, I believe, because over the centuries thousands have stated equally assertively that the fairer sex is also the weaker, both mentally as well as physically. And they have appealed to a whole range of rationales — themselves socially and culturally determined, subject equally to the whims of fashion or assumption — to underpin their preferred judgements. By now no one, I trust, denies that during history women have been largely excluded from scientific institutions (as indeed they have from almost all other institutions) and the mechanisms of exclusion, whether structural, sexual or derived from supposed gender differences, are still largely in operation. Schiebinger's intention is not to add to the burgeoning literature on this topic but to try to understand what these gender differences are, and how they operate in science today. Her chosen method is to examine the past — the revolution in European science in the seventeenth and eighteenth centuries to be precise — in order to answer this problem: what role did the "women question" and the debate over "female nature" play in the origins of modern science?

This is a formidable task, but it has been undertaken by someone of formidable scholarship who is blessed with one additional gift: she understands that social and historical dynamics are highly complex yet has remained undaunted. Halfdan Mahler, when Director General of the World Health Organization, commented recently that "development is a very messy affair" and added "God protect me from the simplifiers". The point applies to this issue too. If we are to make any progress at all in understanding the present inequities, let alone rectify them, we need to eschew simplicity and generalizations and do full justice to the underlying human complexity.

Accordingly, the author approaches her analysis from four different angles but always with the question of gender to the forefront — that is, are men and women different by nature or by nurture; if so, what difference does (and did) this difference make? She explores in turn, first, the institutions of science which in the period under discussion were fostered in a variety of settings (academies, Parisian salons, princely courts and craftsmen's workshops); second, the lives of some women, predominantly from the aristocracy and the crafts, who pressed against the boundaries of convention to practise science; third, how the biological sciences traditionally merged sex (a matter of genes) with gender (a matter of culture, education and expectations) to produce supposedly scientific readings about the female nature that were used equally by those who argued for, or against, women's full participation in science. Finally, her gaze is directed towards the cultural meanings of femininity and masculinity, and how a woman's ability to do science has seemingly become irreparably meshed into issues of gender.

Mary Evans Picture Library

Labour pains — midwives became auxiliaries in a male-dominated medical system.

No one of these elements is, she rightly insists, a causal agent that alone determines where women are in science today. Each, however, had a part in the process, and in the dynamic that determined the present outcome they have all played important and interdependent roles.

The extent of research and the material uncovered is remarkable. Where and how did Schiebinger find it all? For this is intellectual archaeology at its very best, and a landscape is laid out for us the existence of which most readers will be totally ignorant. There are some surprises along the way: matters were not totally ever thus.

Although the extent of women's activity, success and penetration into science varied from class to class and country to country, their ultimate exclusion was not a foregone conclusion. Aristocratic women dominated the discussions in the salons of seventeenth-century Paris; women artesans did notable astronomical and agricultural research in Germany; the Enlightenment was a great time to be alive, when women's desire to be involved in science was assisted by many social trends and even the bastions of some of the new scientific academies were temporarily breached. Yet time after time all such efforts were stymied and it is ironic that the ultimate *coup de grâce* was to come from the very enterprise that women tried to develop. For modern science, too, finally came to codify a view of women that set the seal on their exclusion.

But there is, of course, more than one way to skin a cat, as the history of midwives illustrates. Since ancient times this 'craft' group had peacefully co-existed alongside medical men, each with their own art and monopoly. But both the peace and monopoly were to be shattered in the two centuries in question. Whereas doctors, surgeons, apothecaries, dentists and veterinarians gradually transformed themselves into self-governing, self-regulating professions, midwives never succeeded in doing this though they certainly tried. But still they remained regulated by men — by the King's chief barber–surgeon in Paris, by the clerics in England. Finally, the *accoucheur* appeared — the man-midwife who was now called in for routine cases, not just abnormal labours. By the end of the eighteenth century, midwives were just auxiliaries to a male-dominated medical system: the practice of obstetrics and gynaecology passed over to men.

This example is telling for it illustrates the nature of one of the Catch-22 traps that ensnared so many women who wished to progress in science. Midwives could not learn new methods or practices because they could neither attend universities nor establish their own medical colleges; they could not do those things simply because they were women. No matter what arguments they employed, what authorities they quoted — from the Bible to the new ethnology — to try to secure their ancient privileges, it was hopeless. And when finally they were driven to the argument of 'natural propriety' matters were made even worse. For 'natural innateness' was the final appeal that, above all, kept the women at the hearth in their traditional child-bearing roles.

In giving this account of the role of science in the creation of gender, backed by this mass of fascinating detail, intriguingly interpreted, Schiebinger has truly travelled uncharted territory. But has she really answered her own question? Societies "intent on preserving the sharp social and intellectual distinctions between the sexes" have, for the most part, deliberately cultivated perceived sexual differences. But what are these? Do they indeed exist; if so, what difference does the difference make?

Here these questions are posed rather than answered. Unravelling the multifarious strands of women's role in the history of modern science is an essential first step, brilliantly executed in this book. It is to be hoped that this study will provoke further analysis into the contemporary scene and present powerful trends in science. For every Maxine Singer, now President of the Carnegie Institution of Washington, there are still hundreds of others striving to make their mark in the scientific enterprise in conditions where Catch-22 traps still operate. Will it be ever thus? Sometimes I'm optimistic, but reading what happened in history, and not yet discerning overpowering new trends, sometimes I wonder whether such optimism is justified. ☐

June Goodfield is at The Manor House, Alfriston, Polegate, Sussex BN26 5SY, UK.

Asking questions

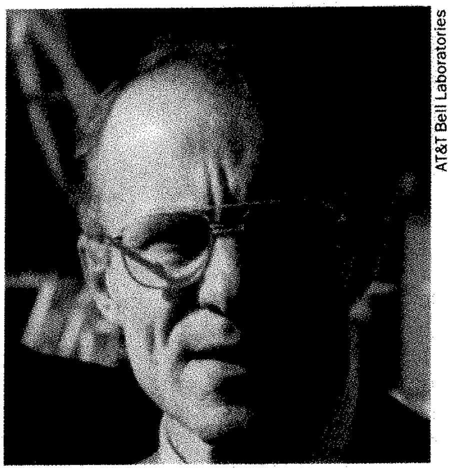
Igor Aleksander

Ideas and Information: Managing in a High-tech World. By Arno Penzias. *W.W. Norton: 1989. Pp. 224. $17.95, £12.95.*

For many people, phrases such as 'the information age' or 'high-tech world' evoke feelings of the hackneyed, the oversold. Not so for Arno Penzias, who sees 'managing in a high-tech world' as a matter of exciting discovery and wonderment. In *Ideas and Information*, Penzias, a Nobel laureate for physics in 1979, glances over his own specialist fence at the nature of information technology and the effects it has had on him and the world he inhabits. The world 'managing' in the subtitle is used in the sense of 'coping': Penzias is a technology enthusiast. His book is intended to give heart to those who feel that coping in a world of faxes, electronic mail, word processing, databases and the like is getting beyond them.

The style is anecdotal without being trivial, authoritative without being overpowering. And it *has* to be enthusiastic because, after all, Penzias is the vice-president for research of one of the world's most renowned information-technology companies: AT&T's Bell Telephone Laboratories. The anecdotes add fun and a personal touch to what could so easily otherwise have been yet another dull catalogue of the benefits bestowed on mankind by the advent of computers. Penzias also goes to great lengths to distinguish the rule-enslaved computer from the free-learning, exploration-loving human being. I suppose that there are still those who get the two confused to the extent that such descriptions are necessary.

The book is written with an implied sense of history: the history of symbols, the history of mathematics and the history of computing machines. Penzias argues that the manipulation of symbols distinguishes computers from other machines in the way that being able to use symbols distinguishes human beings from animals. Symbols were valued by the prehistoric painters of the images in the Lascaux caves in France, by the Sumerians of 3300 BC who invented information storage (tokens in a clay jar), and by the Egyptians and the Chinese, who raised application of their intelligence to higher planes by inventing numbers and mathematics. So, for Penzias, the computer is the ultimate celebration of human intelligence: a machine that embodies the finest points of the use of symbols and manipulations.


AT&T Bell Laboratories

Arno Penzias — "cheerful optimism".

He does not confuse person and machine, however, as seen in this almost thrown-away footnote: "As we explore the computer's ability to match human information-processing capabilities, we shouldn't overlook the fact that these machines have much to offer society without emulating humans". At the same time as he creates the distinction between living and mechanical information processors, he pinpoints the very factor which, if mechanized, could reduce this crucial distance: "On the other hand, I am sure that this situation will change drastically when (and if) we can devise a computing architecture that learns from examples, the way humans do, rather than merely obeying instructions. Nature produces countless billions of such computers in the brains of living creatures". In these sentences he has identified (perhaps unwittingly) the scent that the contemporary hounds of neural-network research have sniffed out, and that is leading them towards learning computers with which they will try to revolutionize computing by narrowing the gap between the mechanical and the biological.

The mix of anecdote, history and insightful comment is a characteristic of the book as a whole. Penzias describes milestones in the making of information machines such as Chomsky's analysis of language, Hopfield's revival of neural nets and Joseph Weizenbaum's totally mechanical 'psychiatrist emulator', Eliza, which makes its interlocutors think that it 'understands' their problems, while it is simply throwing their own words, modified by a few simple rules, back at them.

For me, a high point is reached in Penzias's recounting of I.I. Rabi's description of the reasons for his (Rabi's) success, also crowned by the Nobel prize in physics. "Have you asked any good questions today?", his mother would ask him on his return from school. This, surely, rather than just the ability to learn, must be the factor that best distinguishes organisms that possess intelligence from those that do not. The ones that do ask very good questions.

There are some unfortunate blemishes. Bell Labs is presented as the source of all discovery, which will irritate readers who happen to know that not all high-tech inventions were made at that institution. For example, the comment "At Bell Labs . . . a robotics research group has built a robot that can follow spoken instructions" is followed by a description of work that was done by Terry Winograd at the Massachusetts Institute of Technology in the early 1970s. There is no mention of Winograd. Even Hopfield is described as a Bell Labs' physicist, giving no credit to the fact that he is also an academic at Caltech.

But perhaps the worst sin is that there is no mention of the down side of computers: the overrating of technology for its own sake; the fact that the purchase of information technology in a badly managed organization can amplify the bad management; the need to change one's behaviour to meet the needs of computer-driven procedures (as in banks); new opportunities for fraud; the possibility that people may be forced to become less skilled as well as more skilled. For Penzias these difficulties do not exist, or if they do he may have decided to ignore them for fear of damaging the up-beat tempo of his book. Without a doubt those who need reassurance about coping in the information age will be rewarded by the cheerful optimism purveyed in *Ideas and Information*. Why spoil it with bad news? ☐

Igor Aleksander is in the Department of Electrical Engineering, Imperial College, London SW7 5BT, UK.

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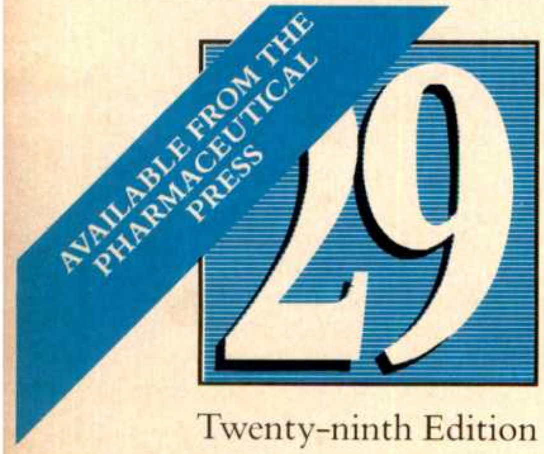
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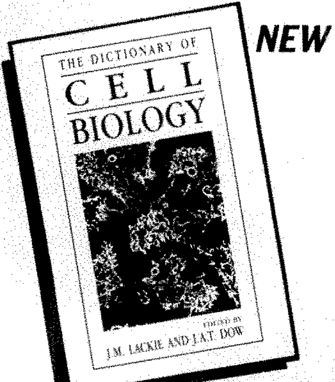

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After the two cultures

Adam Kuper

Academic Tribes and Territories: Intellectual Enquiry and the Cultures of Disciplines. By Tony Becher. *Open University Press: 1989. Pp.200. Hbk £27.50, pbk £9.95.*

Tony Becher's enquiry into the mores of academics is a belated reaction to C.P. Snow's division of intellectual life into two great camps, arts and science, which (if they ever existed) have now long been abandoned for smaller, more intimate, redoubts. An anthropologist, Clifford Geertz, has pointed out that "most academic communities are not that much larger than most peasant villages and just about as ingrown", with their characteristic totems, initiation ceremonies and ancestor rites, modes of work, codes and systems of authority. Professor Becher pursues Geertz's suggestion that these societies should be treated rather in the way that anthropologists study exotic tribes, as social units that have distinctive cultures.

For his enquiry he selected 12 disciplines — biology, chemistry, physics and mathematics, representing the pure sciences; mechanical engineering and pharmacy, as applied sciences; economics and sociology, from the social sciences; history and modern languages from the humanities; and law, representing the social and administrative professions. It is immediately obvious that these categories are not watertight (mathematics, economics and history could all be reclassified), and Becher's thesis is in fact that the most significant criteria for classifying academic pursuits cut across not only the science–arts divide but also disciplinary boundaries, and that they define most clearly the subdisciplines which we call specialisms. This is where the academic has his true being.

Becher's main criteria are familiar: hard versus soft, and pure versus applied pursuits. Hard specialisms use quantitative rather than qualitative methods, clearly define problems and deliver cumulative additions to a body of knowledge. They also tend to be free of value conflicts. Soft specialisms use qualitative methods, address diffuse issues which are often dictated by social concerns (and therefore invite political controversy) and they do not usually yield a body of uncontested knowledge. A more original distinction is that between 'hard, applied' specialisms, which proceed by trial and error, and deliver products which are assessed in terms of their efficiency, and 'soft, applied' professions which use case law, are evaluated in political and moral terms, and produce protocols rather than instruments. Most interesting is Becher's distinction between 'rural' and 'urban' specialisms, the urban specialism being one in which large numbers of workers, operating in competitive teams, address the same closely defined problems, publishing rapidly and often informally, while the rural worker practises his individual craft in a village workshop, where the postman seldom calls.

By applying these criteria to subdisciplines, Becher captures something of the perceived reality of academic life, where the most sensitive boundaries are often drawn within the discipline. He quotes biologists on biologists — "microbiologists are narrow, but geneticists are lively"; "zoologists are more adventurous and less conventional than botanists" but tend to be "arrogant and overconfident"; botanists "hide behind herbarium cases and hate one another". These stereotypes point to some real differences between specialisms which have to do at once with the type of work involved and with the type of people who choose that sort of work (or who are moulded by what they do).

The cultural peculiarities of the tribe are also a matter of organization. Careers, for example, follow characteristic trajectories in particular disciplines. The phenomenon of burn-out, the scientific menopause, which pushes middle-aged men into administration, or philosophy, is most characteristic of hard, pure specialisms. In soft, pure fields scholars become more expert with time, so that a historian may expect to reach his peak only in his fifties. Publications take a different form in different fields. Soft, pure fields typically require books, while hard, pure fields know only short papers. The former are typically written by one scholar over many years, the latter by teams of collaborators on short-term projects. Soft disciplines tend to be dominated by a small number of Grand Old Men, the hard disciplines are multi-centred, throwing up charismatic leaders with shorter tenure and more rivals.

Of course, all academic disciplines also share in the general academic culture, about which Becher has some useful reminders, starting with one of the universals of academic life, "that nearly everything is graded in more or less subtle ways".

His central premise is that "the main currency for the academic is not power, as it is for the politician, or wealth, as it is for the businessman, but reputation". Academic reputation depends on the quality of publications, but it is also affected by institutional affiliation. Publications emanating from famous laboratories or think-tanks start life with a silver spoon in their mouths. Reputation in turn brings

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power over the careers and reputations of others. Within the political systems of disciplines and specialisms, peer groups may serve as democratic controls, particularly in reviewing, but power tends to concentrate in very small networks.

Suggestive though it is, this short study is nevertheless only a preliminary excursion into the field. Becher restricts himself to élite institutions. His fieldwork was done in Britain and the United States, but he gives an undifferentiated account of the Anglo-American university world, although the national variations are surely very striking. A future study, more sensitive to the national character of academic enterprises, should also consider Dutch, German or Scandinavian universities, with their civil service cultures, or European think-tanks, like the Max-Planck Institutes, on which *Nature* has been publishing some unsettling ethnographic reports. A reading of Pierre Bourdieu's *Homo Academicus,* a dense and original recent study of the French academic system, will convince the foreigner that they do things very differently in France. For the purposes of Becher's argument, it is, for instance, noteworthy that the prestige hierarchy of disciplines diverges from the Anglo-Saxon model, and also that the leading research teams are to be found outside the university sector.

Becher does not draw together his scattered comments on the connections which might be made between the peculiarities of various academic tribes and the nature of the territories they inhabit. Here again a comparative approach would have suggested fresh questions. Are German physicists more like German economists in their career patterns and organizational hierarchies than they are like American physicists? Or is it something about physics, or economics, that is decisive? Professor Becher briefly discusses the different environments of the various disciplines — the market pressures on engineers, the professional demands made of lawyers and so forth. But these also vary between different national political systems and markets, as may be seen from the contrasting views of academic engineering in Britain and in Germany.

One important implication of the study is nevertheless clear. These various enterprises are not readily measured by the same criteria because they differ so markedly (and probably necessarily) in their goals, methods, work habits and relationships to the outside world. Bureaucrats will have to refine their measures of efficiency if they are to do justice to this diversity of academic society. ☐

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Gossip value

Lewis Wolpert

Life Among the Scientists: An Anthropological Study of an Australian Scientific Community. By Max Charlesworth, Lyndsay Farrall, Terry Stokes and David Turnbull. *Oxford University Press, Australia: 1989. Pp.304. Pbk A$19.95. To be published in Britain and the United States next year.*

A MEASURE of one's understanding of other groups is how well you appreciate their humour. This applies as much to scientists as any subset of people, for jokes and wit are a prominent feature of laboratory and institutional life. Yet humour is not mentioned in this book. The scientists at the Walter and Eliza Hall Institute of Medical Sciences in Melbourne — the group considered — may of course be unusual, but a more likely explanation is that the social scientists who carried out this anthropological survey simply failed to understand much of what they were studying.

Associated Press

Macfarlane Burnet — Nobel winner.

The authors, who have adopted the curious convention of writing in the first person, like a conflated committee, set out with the provisional hypothesis that scientific knowledge is socially 'constructed'. This is a fashionable view among many sociologists of science and implies that science has rather little to do with the real world that scientists study, but instead is shaped by a complex set of social factors. This relativist stance is then used to argue that scientific knowledge has no particular virtue or validity. These authors take a more equivocal position but basically stick to this idea, and the possibility that science provides understanding of natural phenomena is virtually ignored.

The Walter and Eliza Hall Institute is very distinguished, its contributions to science covering a wide range of topics in molecular and cellular immunology. The first director (1944–1965), Sir Macfarlane Burnet, won a Nobel prize for his clonal selection theory and in the book there is an attempt to view him as a local hero whose social function is to legitimize a particular conception of science — the idea of an individual scientist making a discovery which revolutionizes the field. Burnet was hostile to molecular biology and his general style is contrasted with that of the current director, Sir Gus Nossal. But the analysis provides no insight into how Burnet came to make his great discovery or into the basis of his antipathy to molecular biology. It may not have been his dislike of complex apparatus and expensive equipment, as Charlesworth and his colleagues suggest, but rather his inability to see the power of molecular biology. He simply did not believe that it would be possible to understand cells in molecular terms — they were, for him, impossibly complex.

One of the main conclusions of the study is how large a part 'power' plays in the scientific process, for example in what the authors call the legitimization of molecular biology. They are persuaded that Foucault's insight is "profoundly true", namely that the establishment of specific discourses — ways of speaking and thinking — also provides access to power and control over the objects of those discourses. For them "the new model that came to dominate biology after the discovery of DNA only became established after a good deal of politicking and propaganda", in which 'public relations' used by scientists had a large part. The brief analysis of this period is most unsatisfactory and fails to take into account the change in paradigm from metabolism to information. The authors also do not understand the revolution molecular biology caused, and instead concentrate on the idea that molecular biology was a philosophical movement based on the conviction that all biology could be explained in terms of physics and chemistry. Thus it is said that Crick used Schrödinger's name to legitimize his reductionist approach. Of course there was evangelizing and politicking but none of that would have been effective had molecular biology not provided fundamental new insights. Moreover, scientists are aware of these issues and the nature of scientific politics, fashions and power. That's what they often gossip about.

There is a touching, but nevertheless irritating naivety to the conception of science purveyed in the book. For example, the authors are apparently appalled by "the contradiction between the ideal norms of science, that scientific knowledge should be disinterested . . .

and the spirit of competitive individualism", and imply that competition between scientists (the " 'winner takes all' ethos") is something new. Yet one of the rewards in science has always been priority in discovery — are Charlesworth *et al.* really unaware of the great historical controversies, such as that between Newton and Leibniz over who discovered the calculus? And where do these 'ideals' and 'norms' come from? These really important social constructs are neither questioned nor analysed.

Competitiveness is the subject of a section of the book dealing with immunoparasitology and the quest for a malaria vaccine. Nossal's refutation of their views, which they quote at length, seems to have made no impression. Nossal recognizes competitiveness but emphasizes the tremendous amount of collaboration that science involves. "Now the question is, does the winner take all? Of course not! The peer group is not stupid. . . . The second or third in the race may not win the Nobel prize, but there are plenty of important subsidiary rewards".

This book was intended to convey a sense of the "life-world" of a group of scientists as well as some idea of how that world is "constructed". In this it fails. Much of the description is like that found in an annual report, and any half-decent journalist would have done a far better job. Rather, its value lies in what it reveals about anthropology and social science. Did the authors bring any new insights or techniques from their discipline? There is hardly a word about how the study was carried out or over what period. Did they listen to the gossip? All sorts of important questions are not touched upon. How, for example, does the Institute deal with scientists that are no longer productive; how are resources distributed; to what extent is the research directed; were there different styles of sciences; what are the rewards that scientists seek? An apparent desire for objectivity seems to have killed any curiosity, and any interest.

What, for example, can one say of the following? "What impresses me more and more about a good deal of science at the Institute is the concentration on 'getting data' " and "the idea of data generation seems to me to be a crucial feature of science and is often overlooked. The laboratory is a kind of data factory". No one should be surprised that data are important for biologists, while the metaphor of a "data factory" is plain fatuous. If this is all that anthropology has to offer for studying other groups, then the result is depressing. ☐

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Cut in production

Norman Myers

Tropical Forests: Botanical Dynamics, Speciation and Diversity. Edited by L. B. Holm-Nielsen, I. C. Nielsen and H. Balslev. *Academic: 1989. Pp.380. £29.50, $49.50.*

Tropical Rain Forest Ecosystems: Biogeographical and Ecological Studies. Ecosystems of the World 14B. Edited by H. Lieth and M. J. A. Werger. *Elsevier: 1989. Pp.713. Dfl.475, $250.*

The Fate of the Forest: Developers, Destroyers and Defenders of the Amazon. By S. Hecht and A. Cockburn. *Verso, London: 1989. Pp.256. £16.95, $24.95.*

Saving the Tropical Forests. By Judith Gradwohl and Russell Greenberg. *Island Press, Washington, DC/Earthscan, London: 1989. Pp.214. Hbk $24.95; pbk £6.95.*

WE ARE being overwhelmed by books on tropical forests, some of them less than whelming. The rush to publication reflects the recent burst of interest in the forests as we become aware of their impending demise and their intrinsic wealth.

One of the reasons why we have not done a better job of safeguarding the forests is that we understand all too little of what we are dealing with — at least as compared with savannahs, grasslands, temperate woodlands and tundras — or of what is at stake. There is still only a sparse selection of solid scientific writings on the forests' makeup, their biotic richness, their ecological complexity, their community structures, their dynamic interactions and — above all — their relationships to the human enterprise. For example, we have only a vague notion of how biotically diverse the forests are. Just this year Professor Steve Hubbell of Princeton University revealed that a 50-hectare plot in Peninsular Malaysia contains over 800 species of woody plant, or as many as in all of North America. And when we finally gain a true grasp of the forests' ultimate richness, we shall finally gain a true realization of how far we have to go before we understand their diverse interactions.

Tropical Forests: Botanical Dynamics, Speciation and Diversity provides an abundance of the types of data and analyses that must underpin programmes for conservation and sustainable use. The opening section on forest dynamics appraises the physical factors such as soil and water regimes, together with patterns of tree distribution, gap-phase dynamics, canopy composition and dynamic stability of forest structures overall. There follow extended sections on speciation and diversity, primary and secondary formations, and community dynamics, before the book winds up with a retrospect-and-prospect summation. All of the chapters are authoritative to a degree — among the contributors are Gentry, Halle, Hartshorn, Mori, Oldeman, Polhill and Raven — and they challenge the reader with the thought that tropical forests remain virtually a terra nova to modern science.

Tropical Rain Forest Ecosystems, published in Elsevier's *Ecosystems of the World* series, reflects the experiences of another lengthy list of luminaries — Bruenig, Ewel, Golley, Janzen, Jordan, Kartawinata, Kira, Prance, Sanchez and several others. The book's structure is similar to that of the first. It starts with the physical environment (notably climate among other limiting factors), moves on to tree architecture, taxonomy and classification, then to accounts of interactions such as epiphytism and symbiotic relationships, which lead into a consideration of some plant categories such as lianas, bryophytes and lichens, as well as of mammal categories such as primates, bats and flying insects. These expository reviews are interspersed with assessments of the three main tropical forest regions, and of a number of the countries concerned.

These two books make plain how few scientific answers we yet possess, and how far we are from even asking all the right questions about tropical forests. Regrettably and remarkably, however, neither of them says much about the actual rate at which the forests are disappearing, even though there is now a host of remote-sensing and other inventorying technologies that can provide the relevant data. I estimate that during the 1980s deforestation has accelerated by roughly 50 per cent, not only in Brazil but in Bolivia, the Ivory Coast, Burma, Thailand, Eastern Malaysia and the Philippines. Incidentally, the United Nations agency charged with responsibility for tropical forests, the Food and Agriculture Organization, asserts there has been no evidence of any increase in the deforestation rate during the 1980s — and it does not intend to publish a revision of its 1982 report before 1992 at the earliest.

Nor do the two books attempt any analysis of why the forests are disappearing so rapidly. The editors might legitimately claim that they are natural-science assessments. All the same, it seems strange that in 1989 they should include no multidisciplinary evaluation of the whys and wherefores of deforestation — the economic, political, social and institutional factors at work. One could argue that it is partly because of this deficiency in the past that the forests are now disappearing; ecologists and economists have been far too reluctant to make common cause in presenting an integrated assessment of what the forests amount to and hence how they can be safeguarded.

All the more welcome, then, is the third

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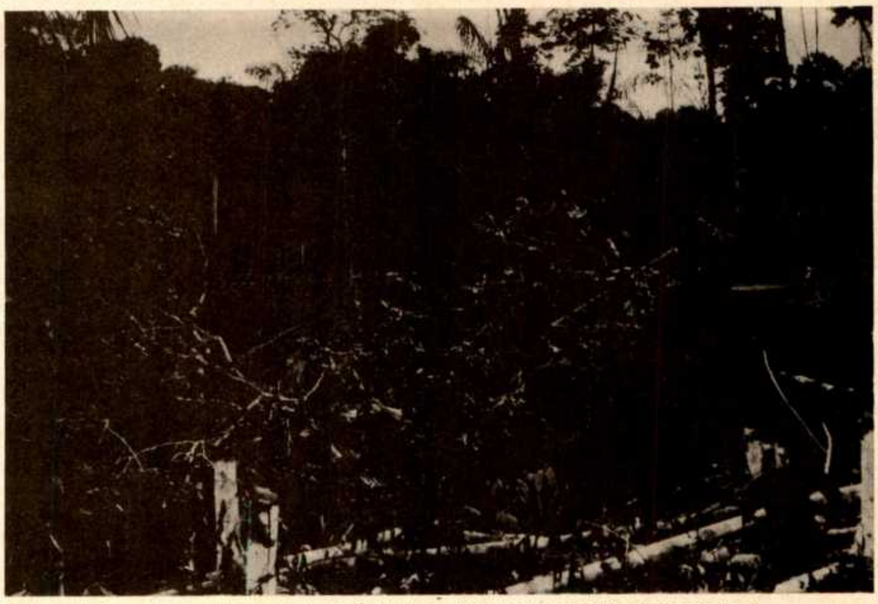

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Felled rainforest — making a fast profit, but leaving greater wealth unrecognized.

book under review, *The Fate of the Forest: Developers, Destroyers and Defenders of the Amazon*. Confined to a single, though the largest and most controversial sector of the biome, many of the authors' insights and conclusions apply elsewhere. The true wealth of the Amazon is little appreciated by the region's political leaders; on the contrary, they often view the forest as a barrier to progress (a term they rarely if ever define). Moreover the recent outbreak of destruction is a departure only in scale. For hundreds of years there have been hands ready to wield the machete and matchbox, and to make a fast profit from just a few forest products while disregarding the many others that could offer a greater return — and do so sustainably.

Hecht and Cockburn's historical perspective is illuminating, and they are to be congratulated on their dispassionate appraisal of the forces bent on deforestation. Salvation for the forest will not depend so much on what goes on inside it, still less on the efforts of conventional foresters. It will come from the land-use planners, economists and development technocrats who operate at the macro-level of national policy, and it will reflect the determinants of agriculture, population, urbanization, financial stability, social equity and political security. These factors will have far greater effect than will logging methods, silviculture, genetic breeding and wood chemistry, or zoning systems, nature preserves and policing efforts. Most important of all, such factors will be determined in places far from the forests, even beyond the countries principally involved, for example in the citadels of foreign investment and the sources of foreign debt.

Finally, *Saving the Tropical Forests* offers a host of specific solutions to the problem declared by its title. The book contains case-studies of 38 examples of success at the local level, covering such broad themes as natural forest management, forest restoration, sustainable agriculture and forest reserves. Among the specific topics addressed are agroforestry, home-garden systems, extractive reserves, watershed management, medicinal plants, multiple-use conservation units, buffer zones and esoteric experiments such as iguana ranching. The book is aimed at conservationists, biologists and policy makers, and presents a fine compendium of recent information on management measures and conservation strategies that demonstrably work — albeit on a small scale — in a variety of economic, sociocultural and political contexts.

But it is a commentary on these four books, excellent within their limitations though they are, and on the traditionalist approach to tropical forests, that none of them ventures a mention of what may soon prove to be the biggest factor of all in the forests' future — the climate connection. It is becoming apparent that tropical forests play a gyroscopic role in regulating climate, whether at local, national, regional or global level. The consequences could be crucial not only for the two billion people who live in the humid tropics, but also for the rest of us. Herein could lie the single most compelling reason for a 'safety first' response to all questions of forest management and conservation. Within ten years, we may well find that the most productive way to exploit the forests is to leave them to get on with their job of maintaining precipitation patterns, temperature balances and the carbon budget of the atmosphere. ☐

Norman Myers is at Upper Meadow, Old Road, Headington, Oxford OX3 8SZ, UK.

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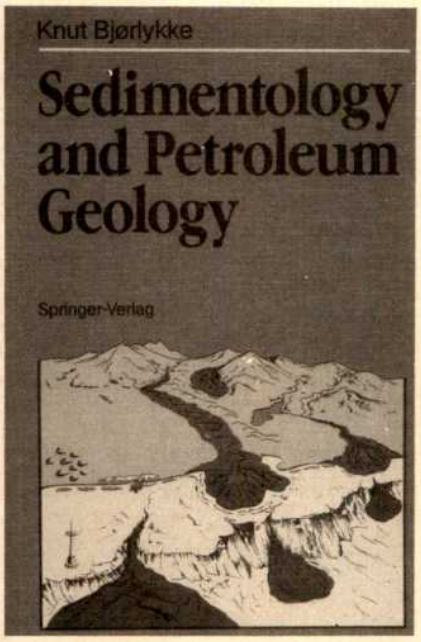

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Predictable outcome

Philip Gummett

Research Foresight: Priority-Setting in Science. By Ben R. Martin and John Irvine. *Pinter, London: 1989. Pp. 366. £35, $45.*

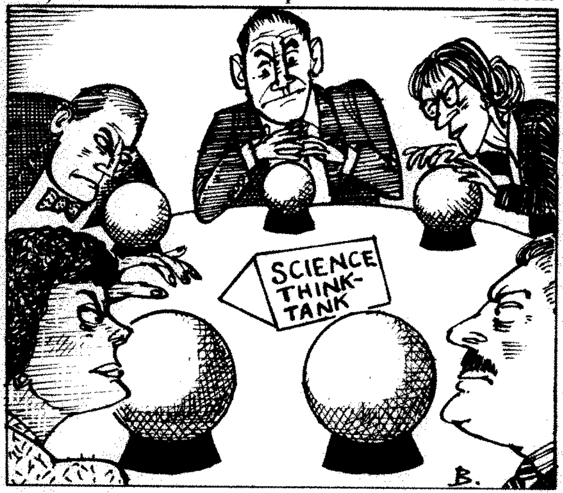
In his *New Atlantis*, Francis Bacon describes the order or society called Salomon's House. This seventeenth-century prototypical research organization operated a well-planned division of labour. Some of the fellows surveyed the literature; others devised or performed experiments; yet others explored the theoretical implications of research results; and some (called 'Benefactors') sought applications of this work. Another important role was performed by those who travelled abroad, in disguise, to collect the books and experiments of other countries. These were called Merchants of Light.

Today, when the state demands increasing returns from its investment in science (so that the 'Benefactors' might be renamed 'Exploiters'), Bacon might think it wise to add a cadre of policy analysts. They would need their own Merchants of Light, for science policy is now an international business. Two candidates might be the authors of the book under review, who have travelled the world, in the guise of contractors to the Dutch government, bringing back information on how eight countries engage in 'foresight' in science and technology. Oddly, having gone to France, West Germany, the United States, Japan, Australia, Canada, Sweden and Norway, they omit their own country, Britain. Their excuse (lack of time and difficulties over commenting on foresight exercises in which they had been involved) is not entirely compelling.

'Foresight' is a slightly curious term. For one thing, what should we call the people who do it? 'Foreseers' sounds too biblical, while 'forecasters' misses a crucial distinction. As the authors explain, the aim is not to forecast, nor — as was suggested by the subtitle of their previous book, *Foresight in Science: Picking the Winners*, published by Pinter in 1984 — to pick winners. Rather, it is to establish a continuous process for reaching an improved understanding of possible developments in science and technology, and the forces likely to shape them, in an attempt to nurture winners and hence create the future.

In their travels, Martin and Irvine encountered a great deal of activity of these types, expecially in relation to strategic and applied research (less so for basic research). There were, though, considerable variations between countries in terms of methods and institutions used, and commitment to the foresight process.

Some countries use formal bibliometric methods to try to spot growth points in science, nationally and internationally. Some engage in elaborate consultative exercises, involving large numbers of scientists and users of science and technology. Some adapt various tools from the kitbag of forecasters and futurologists. Some set up specialist panels on narrow subjects, while others try to take a view of the whole national scene. Some draw on professional analysts (especially Japan, which has a network of some 300 public and private think-tanks, many of them with levels of expertise that are unmatched even by the large US consultancies). Some use what a respondent in the United States called the BOGSAT method (Bunch of Guys Sitting Around a Table).



What lessons can be extracted from this bewildering variety? The Director-General for Science Policy of the Dutch Ministry of Education and Science, in introducing the book, singles out three. First, analysis of long-term needs and opportunities for research should be part of a well-planned process that includes implementation of the choices made. The authors identify a number of attempts at foresight that failed because it was not clear from the outset what was to be done with the results. Second, it is essential, especially for smaller countries, to carry out systematic global monitoring of developments in research. This is something that the Japanese have been doing since the 1950s. Third, and as already indicated above, the aim of policy should be to nurture scientific winners rather than simply to try to pick them, ready-made as it were. That is, a process of identifying, and then supporting, areas deemed to be of national importance should be established.

Other lessons include, first, the point that introducing foresight approaches can be a slow and painful process, especially in countries with no culture of planning or consensus-seeking. This is particularly so in relation to setting priorities in basic research, even in Japan. Second, the authors show the importance of trying to integrate the different levels of the policy process, from all-embracing national socio-economic goals, down through various sectoral institutions and objectives, to strategic and basic research. But they also show the crucial importance, in seeking this integration, of achieving a balance between the views of innovative young scientists, who can see the possible directions in which science might go, and the more seasoned views of senior officials, industrialists and other users, who can relate science-push to social demand, particularly across the boundaries of established disciplines.

From a parochial angle, it is a pity that Britain was excluded from the study. Britain is a country in which formal foresight methods of a bibliometric type have been used by, for example, the Royal Society and the Advisory Board for the Research Councils. The Cabinet's Advisory Council on Science and Technology, and its predecessor, have conducted a number of studies with the intention of guiding policy over research priorities. The University Grants Committee, now the Universities Funding Council, has engaged in substantial restructuring of basic research, though without (as far as one can tell) any vision of the national future that it is aiming to create. And, in response to the arguments in Irvine and Martin's 1984 book, the Centre for Exploitation of Science and Technology — which is mainly funded by industry — has been set up to try to articulate industrial views of the future and integrate them with government policy. The views of our Merchants of Light on these measures would have been welcome.

We can be sure that foresight is here to stay (which may make it worth trying to find a better name for it). The perception is growing that the procedure does not imply draconian planning. Instead it aims at improving communications between producers and users of research and arriving at decisions based on a consensus about the choices to be made. The winners, so the advocates of foresight claim, will be the countries that manage this process best. ☐

Philip Gummett chairs the Board of Science and Technology Policy, University of Manchester, Manchester M13 9PL, UK.

- Recently published by Routledge, London, is *Innovation and Technology Transfer in Japan and Europe: Industry–Academic Interactions*, by Glyn O. Phillips. The book provides "a perspective against which we [the British] can take decisions about the future of our own industry–education interaction arrangements" and costs £45.

Fact and fiction

S.A. Barnett

The Longman Literary Companion to Science. Edited by Walter Gratzer. *Longman: 1989. Pp. 517. £17.95. To be published in the United States by Norton.*
From Creation to Chaos: Classic Writings in Science. Edited by Bernard Dixon. *Basil Blackwell: 1989. Pp. 280. £15, $29.95.*

HERE are two compendia about aspects of the scientific life. Both, especially Gratzer's, can be read with enjoyment; both are highly misleading.

Gratzer wishes to "explore the incursions of science into literature". Happily, his 216 excerpts (each of which has a brief editorial introduction) are not only from stories and poetry; many are highly readable and informative passages from biography and journalism. Readers should not be deterred by the unrepresentative false start. The first section, "The Laboratory Scene", opens with a series of exposés of "the research supervisor as a monstrous incubus". Supervisors are far more often harrassed than depraved. In default of a quantitative survey of supervision, we need a David Lodge to write a hilarious but authentic novel on the struggles of a supervisor to get her pupils to write up their work, and her final resort to writing the papers herself.

After the supervisor, we have "the research grandee and the array of his vassals". Nobel prizes, and their winners, though rare in real laboratories, loom large in this collection. Only by the fifteenth entry do we reach one of the few fictional descriptions of what research is really like: in Nigel Balchin's *A Sort of Traitors,* two research assistants patiently but resentfully record many thousands of cultures as showing "slight growth". And, much later, there is a comment on Ernest Rutherford (in Gratzer's words, "probably the greatest experimental physicist of this century"): "he got pleasure not only from the high moments, but also from the hours of what to others would be drudgery, sitting in the dark counting the alpha particle scintillations on the screen". Another rarity is a poet, Louis MacNeice, with his unromantic but not unkind portrait of a scientist who

. . . lived by measuring things
And died like a recurring decimal
Run off the page, refusing to be
curtailed;
Died as they say in harness, still
believing
In science, reason, progress.

A book such as this could hardly dwell at length on quiet people and non-events. It is appropriate that we should be horrified by the effects of anti-semitism in Poland, or of McCarthyism in the United States; that we should be frightened by an account of accidental death due to radiation; and sickened by the outcome of mindless bombing in the Second World War. In contrast, we may applaud the case of the brilliant but wayward student, admitted to a course of study though without qualifications. And we may enjoy passages from those masters of satire, Jonathan Swift and Samuel Butler, or turn for light relief to stories of the great in disconcerting situations — for instance, Albert Einstein trying to steal tobacco from Niels Bohr.

Gratzer's exertions do not seem to have been entirely a labour of love: he comes over as ambivalent towards scientists. He quotes a tribute by a classical scholar, Maurice Bowra, who said that scientists were treacherous allies on committees because "they are apt to change their minds in response to arguments", but he immediately qualifies it by reference to scientists' "passions" and, in a section on "Discovery", he states that "emulation and jealousy among scientists have [today] become sanctified as the motives that drive scientists ever onwards". Here, perhaps, is another hypothesis in need of a survey.

He finishes, as he begins, with bathos: the last section, "The Pathology of Science", contains a mixed bag of accounts of fraud and plagiarism by scientists and bogus science by illusionists, with only one (fictional) example of commercial skulduggery. Gratzer could have acknowledged that his patchwork is far from a representational portrait of science by ending with a remark by the physicist, Jeremy Bernstein, quoted on p. 151: "I pity the historians of science and others who are attempting to put together a reliable history [of science]; all you have to do, you may think, is ask the participants for their accounts of what happened. But such stories can be woefully misleading".

Capable writer — J.B.S. Haldane at University College, London 1948.

Bernard Dixon, too, is concerned with literature: by publishing his collection, he hopes to "demolish the well-worn cliché that scientists, by their nature and training, cannot write", although his medley of excerpts includes many by science writers (and even a rather dim limerick by Arthur Koestler). Most of his preface is directed at encouraging young scientists to write well. He warns adolescents against trying to imitate "models of unattainable excellence", and says that instead they should learn by analysing bad writing and read great prose for pleasure so that "lessons of style . . . sink in by subconscious osmosis". But he ignores the principal, perhaps the only, effective method of developing order, clarity and conciseness, which is to practise and to subject one's attempts to criticism.

Dixon also tells us that his book's "sole *raison d'être* is to provide pleasure for the reader". Except that they are said to be selected "solely on the basis of literary quality" the writings are unfortunately presented without editorial comment. Yet the pleasure and instruction one derives from reading are due to the combined effects, often inextricable, of style, content and context.

After the confusing preface, a reader should not be surprised to find a rather uneven selection. The passages are arranged alphabetically, hence are randomized. Six items are in verse. Of 69 prose excerpts, 18 are fragments that convey little and hardly do justice to their authors. They could well have been replaced by longer passages from writers such as Bertrand Russell and Joseph Needham who are not represented.

Among the remainder are several which, I hope, will send readers back to the originals. Here is Haldane, in 1932, on the evolutionary process: "In the first place, it is very beautiful. In that beauty is an element of tragedy. . . . In an evolutionary line rising from simplicity to complexity, then often falling back to an apparently primitive condition before its end, we perceive an artistic unity similar to that of a fugue". And here is Peter Medawar (1960), another polymath, writing less agreeably on the same subject: "It is a profound truth . . . that nature does *not* know best; that genetical evolution, if we choose to look at it liverishly instead of with fatuous good humour, is a story of waste, makeshift, compromise and blunder".

Human evolution appears in questions asked by A.R. Wallace (1875) in an unfamiliar idiom: "whether we compare the savage with the higher developments of man, or with the brutes around him, we are alike driven to the conclusion that in his large and well developed brain he possesses an organ quite disproportionate to his actual requirements — an organ that seems prepared in advance, only to be fully utilized as he progresses in civilization".

Wallace also appears in an extract from Darwin's *Autobiography* of 1876, where the author scolds himself for overlooking (in the 1840s) an important problem — "the tendency in organic lines descended from the same stock to diverge in character as they become modified. The solution, as I believe, is that the modified offspring of all dominant and increasing forms tend to become adapted to many and highly diversified places in the economy of nature". An alternative autobiographical model is provided by Otto Frisch (1979), who describes a visit to his aunt, Lise Meitner, in exile in Sweden, which led to a major discovery in atomic physics. Or one may turn to H.G. Wells (1934) on his unsuccessful attempts, as a hamfisted student in the 1890s, to make apparatus, and his all too successful campaign to burlesque his professor; or to Miriam Rothschild writing cheerfully, in 1987, on having seven years of work destroyed by a Nazi bomb.

The reader may also compare T.H. Huxley's famous account of a piece of chalk (1868) with Primo Levi's still more readable story of a carbon atom (1984). Rachel Carson's brilliant *Silent Spring* (1962) may be contrasted with E.J.H. Corner's lucid *Life of Plants* (1964). A problem outside the natural sciences is presented by S.J. Gould (1984) in a discussion of Adam's navel — an enigma indeed, for Adam was created, not born. These and other passages show that a few people with scientific training write well, even superbly. Classified and put briefly in context, they could have made a treasury to be read both for pleasure and for instruction. I especially emphasize the second purpose, for such a volume is needed to help in remedying a lamentable state of affairs — that most scientists still write badly. ☐

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Blaming biology?

Daniel J. Kevles

Dangerous Diagnostics: The Social Power of Biological Information. By Dorothy Nelkin and Laurence Tancredi. *Basic Books: 1989. Pp. 207. $18.95.*

BIOMEDICAL research, especially in genetics and the neurosciences, has been generating an increasing range of new diagnostic methods and machines. Medical genetics can specify several thousand single-gene diseases and disorders, while magnetic resonance imaging, PET-scan and other techniques can detect numerous abnormalities in the brain. The new diagnostics reaches beyond the detection of physical maladies such as Tay–Sachs disease or brain tumours to identify tendencies to particular types of behaviour or mentality, drawing on recent scientific research that has found, for example, genetic sources for schizophrenia or brain lesions linked to learning disabilities. To be sure, the growth in diagnostic power has far outpaced therapeutic capacity. But never mind: diagnosis has its uses independent of cure. Diagnosis produces information about individuals that can assist life decisions they make for themselves — or that are made for them by others.

Genetic assessments have for some years figured in clinical settings, typically permitting prospective parents to learn the risk of conceiving a child that suffers from a genetic disease or assisting the physician in identifying the physical cause of a patient's malady. However, the broadening powers of neurophysiological and genetic tests are making them evermore appealing as devices to screen individuals with benefit, it is said enthusiastically, not only to themselves but to society. The National Institute of Mental Health has expressed a commonplace expectation: "Pre-symptomatic detection of psychiatric disease will be routine. Subjects at risk for alcoholism, schizophrenia, or depression may be identifiable well before the onset of clinical symptoms" (pp. 5–6). Families and physicians will thus be better prepared to take preventive action or apply therapeutic measures.

Diagnosis is similarly touted for use elsewhere in society. In the schools, it will assist teachers in dealing with troublesome children; in the courts, help specify the degree of a defendant's mental capacity; in the insurance world, aid in the determination of a client's health risk and, hence, premiums; and in the workplace, identify the propensity of workers to fall ill in response to, say, chemicals. According to the *Journal of Occupational Medicine,* diagnostic screening makes good business sense because it can "lead to reduced absenteeism, increased productivity, and decreased expenditures for workers' compensation and group health insurance" (p. 90).

In *Dangerous Diagnostics,* a critical survey and analysis of the diagnostic trend, Nelkin and Tancredi allow that the testing may indeed be valuable — as is, for example, DNA fingerprinting in the courts — but they argue, with arresting persuasiveness, that much of it is alarming. Nelkin, a sociologist at New York University, is an authority on the affairs of science and society. Tancredi directs the Health Law Program at the University of Texas Health Science Center in Houston. Their respective talents and expertise are advantageously joined in this book, which they wrote to stimulate debate about "the potential uses and abuses" of the emerging biological tests and as a kind of technology assessment addressed to their inherent social and political implications (p.ix).

Among the strengths of Nelkin and Tancredi's treatment is their insistence that the danger in diagnostics arises not so much in any evil intent as in the way that institutional contexts, and the values inherent in them, encourage the assessments to be used — and abused. A rapidly growing diagnostics industry presses the tests upon the world ("The future lies in genetic tendencies", an industrial biotechnologist has declared – p. 5). In the courts, reliance on 'hard' biological evidence of mental condition may improve the efficiency and reduce the costs of the judicial process; it can also exclude traditional psychiatric testimony that places the mental state of defendants, to their advantage, in a broader setting. Diagnostic screening in the workplace may improve productivity and decrease liability; it also shifts responsibility for workplace hazards from the company to the employee. Screening may better reveal an individual's risk of disease; but it enables health-care providers and

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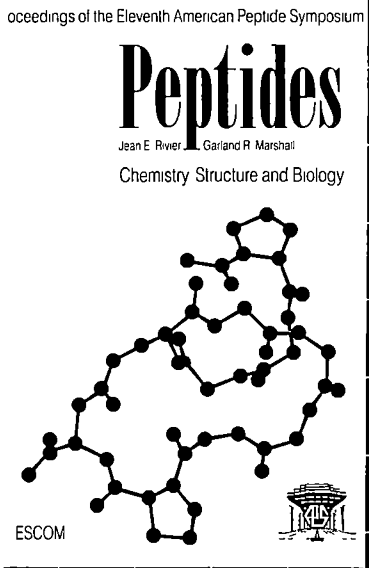
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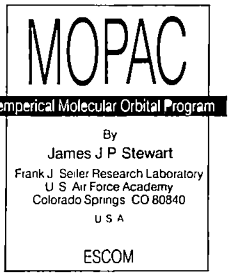
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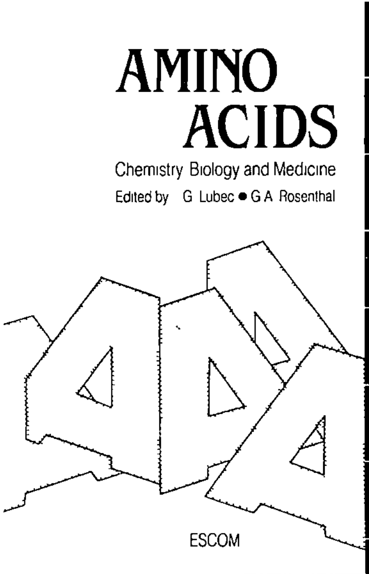
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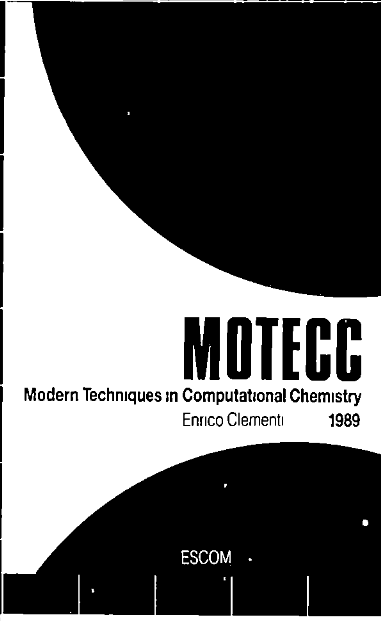
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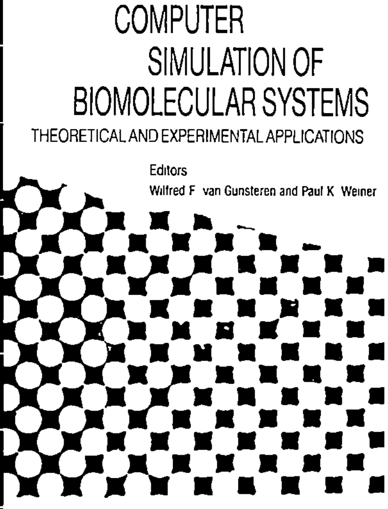
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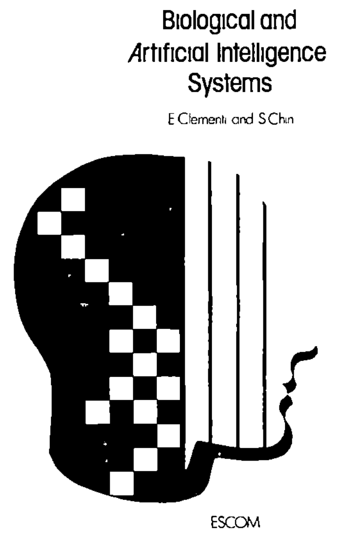
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A strange type of addiction

David L. Hull

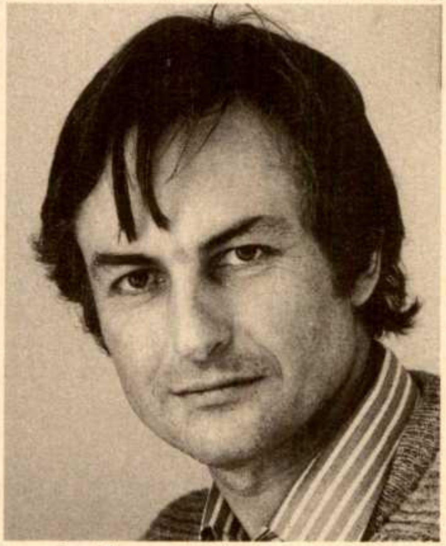
The Selfish Gene, new edition. By Richard Dawkins. *Oxford University Press: 1989. Pp. 352. Hbk £17.50, $22.95; pbk £5.95.*

The Selfish Gene appeared in 1976. Dawkins wrote the book to educate general readers about biological evolution, to prod experts out of their slumbers over the issue of individual selection and to catch the imagination of students. He succeeded on all counts. For this second edition, he has not engaged in rewriting on a large scale. Instead he has added two chapters at the end and interspersed his text with footnotes in which he acknowledges errors, expands on certain points and replies to his critics. The result is a more moderate and conciliatory book than the original. Dawkins has caught our attention. Now he can afford to present his views in more measured terms.

One of the issues that has fascinated Dawkins is exactly how nice we can be given the constraints placed on us by biological evolution, in particular the 'selfishness' of genes. He is not impressed by objections that genes can no more be selfish or unselfish than atoms can be jealous. Scientists have always borrowed words from ordinary language and transformed them into technical scientific terms. If Newton could use the Latin equivalent to 'force' to characterize mass, and quantum physicists can talk about a subatomic particle having 'charm', then biologists are justified in turning 'selfishness' into a technical term as well. Unfortunately, some choices can lead to misunderstandings — the selfishness of genes in the technical sense has consequences for the selfishness of organisms in a more ordinary sense, and the two are easily confused.

Dawkins is swayed more by objections that turn on the social implications of his way of expressing himself. He began working on *The Selfish Gene* in 1972 when power-cuts resulting from industrial strife forced him to interrupt his laboratory research. At the time the behaviour of the working classes in Britain seemed to offer increasingly good examples of selfish short-sightedness. But times have changed: "Now that Britain has a government of the new right, which has elevated meanness and selfishness to the status of ideology, my words seem to have acquired a kind of nastiness by association that I regret" (p. 268). He also regrets his overly cynical and selfish view of life expressed in his chapter on the battle of the sexes, but at the time he felt the need to counteract the Pollyannaish view then prevalent of the relation between the sexes.

In the original book, Dawkins acknowledged that a "human society based simply on the gene's law of universal ruthless selfishness would be a very nasty society in which to live". In part he intended to warn us that if we want, as he does, to "build a society in which individuals cooperate generously and unselfishly towards a common good, you can expect little help from biological nature" (p. 3). Our only hope for a better society is to understand our biological make-up and to counteract it. As surprising as it might sound coming from someone who has so strongly emphasized the role of genes in biological evolution, Dawkins nevertheless insists that "Darwinism is too big a theory to be confined to the narrow context of the gene" (p. 191). Genes function as the replicators in biological evolution while memes serve this same function in cultural evolution.



Dawkins — catching our attention.

Early darwinians such as Huxley presented their contemporaries with the choice of either darwinism or miracles. Dawkins seems to be presented with a comparable dilemma — either genetic determinism or free will. He insists that he was not and never has been a genetic determinist. Genes exert the same sort of statistical influence on behaviour as on other phenotypic traits. Genes are necessary. They are never sufficient. The influence of genes can be "modified, overridden or reversed by other influences" (p. 331). These other influences are to be found in our environment, especially in our society. "We, that is our brains, are separate and independent enough from our genes to rebel against them" (p. 332). The crucial issue is our power to influence which memes come to reside in our brains. Memetic determinism is no less deterministic than genetic determinism. We can rebel against our genes, but can we rebel against our memes? Science is one way. If science is anything, it is a method that can force us to change even our most cherished memes.

The two additional chapters added by Dawkins are entitled "Nice Guys Finish First" and "The Long Reach of the Gene". The first corrects and expands upon his earlier discussion of evolutionarily stable strategies. If one looks at simple versions of such game-theoretical problems as the Prisoners' Dilemma, it is difficult to see how cooperative behaviour can become prevalent. Nice guys cannot win. But in real life organisms play repeated (or iterated) games. Behaviour that has a low payoff if each game is made up of new players can become prevalent if successive games are played with the same contestants. In iterated games it turns out that the nastier strategies rarely prevail — and selection is inherently an iterated game. Thus, Dawkins concludes that "even with selfish genes at the helm, nice guys can finish first" (p. 223). But notice that genes are still at the helm.

The other new chapter is not concerned with who is nasty and who is nice but with the basic structure of selection processes themselves. For all the modifications that Dawkins has introduced into his theory, he still argues for the primacy of genes, but he now sees more clearly in what ways they are more primary than organisms. In selection processes entities must replicate themselves. They must interact causally with their environments in such a way that replication is differential. Differential replication in the absence of such environmental interaction is drift, not selection. Dawkins terms the entities that perform the first function "replicators". According to him, genes are the sole replicators in biological evolution. Replicators can interact directly with their environments or produce more inclusive entities (vehicles) that perform this function for them.

One of the reasons why Dawkins wrote *The Selfish Gene* was to combat the alacrity with which so many ethologists explained certain behaviours in terms of group selection. "We can now see that the organism and the group of organisms are true rivals for the vehicle role in the story, but neither of them is even a *candidate* for the replicator role" (p. 254). Organisms and even certain sorts of colonies can function in selection processes — as vehicles, not replicators. Dawkins is a genic replicationist, not a genic selectionist.

The part of the second edition that I found most valuable is Dawkins's reworking of the foundations of our understanding of the evolutionary process. All that is necessary for selection to occur is that genes have phenotypic effects. But, in point of fact, genes are organized into genomes, they gang up in cells, cells gang up into multicellular organisms and some organisms gang up in colonies. If discrete,

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individually purposeful vehicles are not necessary for selection, why are there so many of them? And why do multicellular organisms adopt a 'bottlenecked' life cycle?

The crucial distinction for Dawkins is not between sexual and asexual reproduction, but between those organisms that periodically go through cellular bottlenecks and those that do not. In the second case, no difference exists between growth and reproduction. Mutated cells find themselves clumped with distantly related cells that retain the original genetic make-up. But organisms that go through single-celled bottlenecks can go back to the drawing board. Instead of attempting to reorganize an entire organism, all that has to be modified is a single genome. Sequestering certain cells into germ lines facilitates the evolution of complex organisms. This mode of organization seems so advantageous, one wonders why it is not universal. Clonal organisms must be doing something right because they are still with us.

Although Dawkins grants that bare genes are invisible to natural selection, he argues that natural selection acts on genes through differences in their phenotypic effects, and these effects need not stop at the boundaries of our bodies. They extend to all the effects that a gene has on the world, including responses in the behaviour of other organisms. Thus, both Dawkins's book and the responses to it count as part of his extended phenotype. In one place, he describes an adult dunnock feeding a cuckoo chick that it has been tricked into adopting. The dunnock is so much smaller than the cuckoo chick that it must perch on the back of this monstrous foster-child to feed it. Dawkins remarks that German ornithologists refer to such foster-parents as 'addicts' and their cuckoo nestlings as their 'vice'. As I read this description, I pictured him perched atop his theory attempting to feed its gaping mouth. Scientists give every appearance of being addicts, and science is their vice. That is one reason why progress in science is so rapid. I for one have benefited a great deal from Dawkins's addiction. ☐

David L. Hull is in the Department of Philosophy, Northwestern University, Evanston, Illinois 60208, USA.

Facts of life

W. F. Bynum

The Timetables of Science. A Chronology of the Most Important People and Events in the History of Science. By Alexander Hellemans and Bryan Bunch. *Simon & Schuster, New York/Sidgwick & Jackson, London: 1988/89. Pp. 656, $29.95, £19.95.*

Asimov's Chronology of Science and Discovery. By Isaac Asimov. *Harper & Row, New York: 1989. Pp. 707. $29.95, £22.95.*

WHAT are we to make of the almost simultaneous publication of two chronological guides to the history of science and technology, especially when a third — *Breakthroughs: A Chronology of Great Achievements in Science and Mathematics* by Claire L. Parkinson (Mansell, 1985) — has already been remaindered? One conclusion is simply that publishers think such books will sell (despite the remaindered example). More significantly, perhaps, is the fact that scientists do think about the history of their endeavours chronologically. As popularly conceived, scientific knowledge is cumulative, in ways that make it matter that Newton came after Galileo, or Dalton followed Lavoisier. Dates also count in priority disputes, to say nothing of the excuse anniversaries provide for holding jamborees. Who knows, anniversarial studies may even be recognized soon as an important branch of the history of science.

Some of the attractions and pitfalls of this emerging discipline are amply illustrated by the two books under review. Asimov's is the easier to read, because he writes short, discursive essays, mostly of around 200 words, for most of the discoveries or inventions covered. There is something for every year since 1793 and for most years from about 1600. It all becomes rather formulaic: 1902 and 1970 were good years, with eleven entries each, whereas 1958 and 1928 were rather ordinary, with only five. Fleming's discovery of penicillin gets about as much space as Marguerite Perey's discovery of francium, Diderot's *Encyclopédie* about as much as the fifth satellite of Jupiter (Amalthea).

Asimov ends with the greenhouse effect, 4,001,988 years after his first entry, on bipedality, thereby giving a symmetry to the volume — if the first process had not occurred, neither would the last. In between there is much to admire but also everything to take on trust. As befits the author of 425 other books, Asimov has eschewed preface, acknowledgements, references or bibliography. The only personal touch is a dedication.

Hellemans and Bunch (both of whom are science editors) don't tell us where they got their information from either, although in the preface they acknowledge help and provide the useful information that they "have relied upon the consensus of many historians of science whose works we consulted to help locate the events as closely as possible". They record about 10,000 separate items, ranging from a birth or a death to a publication, experiment or invention. Throughout, a 'general' column locates the ordinary events of history (beginning of the English Civil War) or 'non-scientific' aspects of their story (publication of Freud's *Jokes and Their Relation to the Unconscious*, introduction of Oreo cookies by the National Biscuit Company). Their timespan is shorter than Asimov's: they also end in 1988 but don't begin until 2,400,000 BC. The spare, atomistic approach to separate bits of information makes the book impossible simply to read. But a series of nine overviews (for example "Science after World War II") and more than 100 short essays provide some social and intellectual context for entries such as "A. Meissner invents a radio transmitter with vacuum tubes" (1913), "Permanent press clothing is introduced" (1964) or "Ernst Chladni invents the euphonium, a musical instrument in the tuba family" (1789).

The assiduous reader of Asimov would not have picked up any of these latter facts. For Asimov, the key dates for the radio are 1895 (radio antennae), 1901 (Marconi's sending of radio signals from England to Newfoundland), 1904 (J.A. Fleming's rectifier), 1906 (R.A. Fessenden's work on amplitude modulation), 1916 (E.H. Armstrong's superheterodyne receiver), 1939 (Armstrong's perfection of frequency modulation), 1948 (transistors) and 1954 (improvements in the transistor). For Hellemans and Bunch, there are at least 21 key moments, in addition to Meissner's work. For them, the radio antenna did not properly appear until 1905, Armstrong's superheterodyne receiver came in 1918 and Armstrong perfected frequency modulation radio in 1933. These authors agree with Asimov on the other dates, they also add a number of other moments in the history of radio.

Discrepancies also emerge in cross-checking through a year rather than a subject. Take 1922, for instance. Asimov writes of Leonard Woolley's excavation of Ur (dated to 1918 in Hellemans and Bunch, where his name is spelled Wooley); Howard Carter's discovery of Tutankhamen's tomb (confirmed by H and B); Herbert Evans's work on what became known as vitamin E (confirmed by H and B, although with the addition of K. J. Scott); Evans's research on growth hormone (not mentioned at all in H and B); Alexander Fleming's isolation of lysozyme (dated to 1921 in H and B); Oparin's work on the origin of life (under 1936 in H and B); experiments of J. Erlanger and H.S. Glasser on nerve conduction (recorded by H and B under 1944, when the Nobel prize was awarded); and A.A. Friedmann's work on the expanding Universe (confirmed by H and B).

Who would want to be an anniversarial scientist when there is so much uncertainty about the facts of history, to say nothing of their interpretation? Not I, which is why I have steadfastly refused to adjudicate between Asimov and Hellemans and Bunch. It would, after all, simply be my reference books against theirs, and since they have declined to reveal theirs, it would be an unequal contest. I have reason to believe, however, that Leonard Woolley spelled his name thus. ☐

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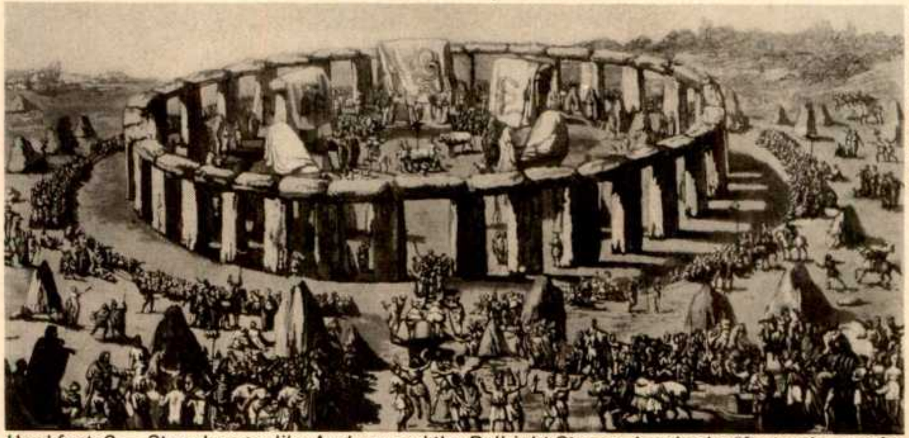
Of thoughts past

Graham Ritchie

Ancient Britons and the Antiquarian Imagination: Ideas from the Renaissance to the Regency. By Stuart Piggott. *Thames & Hudson: 1989. Pp. 175. £14.95, $19.95.*

As excavation techniques extend the range of what can be recovered, and theoretical considerations crowd into university curricula, archaeology increasingly turns to science. How important it is, therefore, that in the history of ideas about early British antiquity we should be aware of the attitudes of early scholars and have an appreciation of the framework within which their thinking took place. Stuart Piggott's perceptive exploration of antiquarian ideas draws together many strands of research to create a fascinating picture of the intellectual worlds of well-known figures such as Aubrey and Stukeley, and at the same time introduces many lesser known writers from the late sixteenth to the early nineteenth centuries.



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Hard facts? — Stonehenge, like Avebury and the Rollright Stones, inspired a "fantastic past for ancient Britain, dominated by fictitious Druids".

The past must be understood within the intellectual constructs of the time, and thus we are not introduced to approaches to prehistory — for the term prehistoric was first used by Daniel Wilson in 1851 — but to a world for which the chronology of early times was outlined in the Old Testament. The early antiquaries are to be seen as individuals undertaking research within their own views of the world. It is a measure of Piggott's success that within this rigorous approach the reader is so pleasurably taken on a voyage of exploration through the worlds of the computation of Biblical chronologies, ancient giants and what happened after the Flood.

The Indians and Eskimos — even a Brazilian king — who were transported from the New World to be paraded before the European public in the course of the sixteenth century had a considerable effect on antiquarian approaches and on the way in which early Britons were depicted. It is startling to realize that at least a thousand Red Indians were brought back to Europe by early voyagers and that Frobisher brought Eskimos back from his expeditions to South Baffin Island in 1576–77. Early antiquaries drew comparisons between aspects of American Indian and Eskimo characteristics and those of the ancient Britons. Ethnographical illustration had a great deal of influence on the way that early man was depicted, and Piggott has assembled an unusual series of illustrations of Eskimos, Britons and, most enchanting, Picts and other North British tribesmen.

The fieldwork and recording of monuments by John Aubrey and Edward Lhuyd are set within the context of the day. Our current understanding of monuments such as Stonehenge, Avebury and the Rollright Stones begins with observations made three centuries ago. Thus William Stukeley is seen on the one hand "as a field archaeologist of great distinction" and on the other as the man who misled generations "as the inventor and propagator of a fantastic past for ancient Britain, dominated by fictitious Druids" (p. 122). Stukeley's fieldwork at Avebury and Stonehenge is the earliest detailed recording of field monuments; between 1719 and 1724 he spent up to two months at a time surveying them using a theodolite, and making notes and drawings. In a way that we think of as 'modern', Stukeley's concern was to put on record monuments at risk from agricultural and building developments. ▶

Piggott sets in context the decline in scientific endeavour in the decades following 1700, and leads us through to the campaigns of barrow-digging in Wiltshire a century later. Some archaeologists today belong to the traditions formulated at that time, and I can feel much sympathy for the "young antiquary, who, from practice, can open a barrow as nicely as you could cut up an apple pye; and from his description, it is done much after the same manner" (p. 156). Archaeologists need to understand how our attitudes to the past have been moulded.

In this volume Stuart Piggott draws together themes that he has discussed in his studies of the Druids, in his collected essays on antiquarianism, *Ruins in a Landscape*, and in his biography of William Stukeley. Here they are put within an historical and cultural setting that is both accessible and authoritative. ☐

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Complex stability

Paul Colinvaux

Thinking Green: An Anthology of Essential Ecological Writing. Edited by Michael Allaby. *Barrie & Jenkins, London: 1989. Pp.260. £14.95.*

In its issue of 23 September 1989, *The Economist* carried a review of the economies of Third World countries. The conclusion was that whether these economies caused disastrous poverty, or wealth like the dragons of Asia, depended only on whether the governments are good or bad, and no mention was made of possible limits to growth on a finite Earth. Writers in the environmentalist 1970s saw things differently. Their concern was for the miserable social consequences of human numbers rising beyond some critical density, or for the fouling of the human nest by industry. Both views are surely right.

But in their anxiety to tell their dreadful truths, the 'green' writers made what Sherlock Holmes called "the capital mistake" of reasoning beyond their data. Their predictions of famines and shortages and limits and catastrophes were not warranted by what we knew. Readers of *The Economist* can therefore ignore them, even as green philosophies begin to mould the politics of the industrial states.

Thinking Green is a first-class anthology of extracts from the sacred texts of the greens, 45 short essays in all. *Silent Spring*, *The Commons*, Malthus, *The Population Bomb* and *Spaceship Earth*; all are here, together with their many supporters and a tiny few of their opponents. The extracts are short, mercifully so in the case of the wearisome establishment rewrites of such documents as *The Brandt Report*, and of *Global 2000* and *Blueprint for Survival*. But the propagandists themselves are generally a very good read. Rachel Carson is superb, standing out even among essays that include Charles Dickens writing on 'Coketown'. This is a book around which to build a seminar for our times.

But the seminar will have to acknowledge much error on the path to truth. Green thinking is riven by longing for a superorganismic Earth that never was. Green writers declared that the complex was stable and the simple not. Almost no modern ecologist will accept this; mathematics, empirical data and the process of natural selection are all against it. Yet the mystical belief in an organic nature not to be trifled with lingers on. *Global 2000* had in its opening sentence the idiot assertion that the coming world would be "less stable ecologically", and this was 6 years after Robert May's demonstration of the falseness of complexity–stability theory. The latest reincarnation of a superorganismic Earth is complete with a name from the myths whence it came, 'Gaia', and is allotted two extracts.

The Mansell Collection

Thomas Malthus — original thinker.

The central catch phrase of Malthus, that food supplies grow arithmetrically whereas populations grow geometrically, has proved meaningless. Food supplies have often been increased exponentially for prolonged periods. And human beings do not breed like animals, but do so under conscious parental choice subtly affected by cultural values. Malthus himself knew this, and in the extract from his *Essay on Population* declaims against the "viciousness" that follows times when men elect not to marry.

Mercifully, green propagandists now also realize that they were grievously misled by those who threatened us with a loss of atmosphere as the wages of pollution, so that none of the extracts in the book include ponderings about our running out of oxygen because of the poisoning of plants in the sea. The editor must have chosen his material with care.

With these essential criticisms in the open, the core of *Thinking Green* describes the true dilemmas of our times. The good life requires access to a diverse nature, people in tolerable densities and freedom of choice, qualities that are not so easily provided as is the 'wealth' that economists measure.

The numbers of people can be contained, if we have the patience of generations, as we must. The curse of excessive families is a cultural curse, not a biological one. We do act to regulate our reproduction, even the poorest of us when settled in stable cultures. If we think with the green writers, as I do, that the sooner we bring the numbers down the better, then there is an easy way. Liberate women completely, in culture after culture, so that female lives are fully equal in opportunity to those of men. Can anyone doubt that women so liberated would, on the average, decide to have fewer children?

Some environmental abuses are solvable by law, although neither the laws nor the methods of enforcement have yet been devised. The tragedy of the commons is being played out in the world oceans as Asian ships with 30-mile nets strip out all large living things; world law and warships could correct this, as they did for a different form of piracy long ago.

The misery of peasants in lands once parts of Spanish and British empires comes in part because productive land was taken from them to grow cash crops to support Spanish and English gentry, a way of life that is retained by their local, gentrified successors. In the English heartland of the industrial revolution, subsidized industry — posing as farming — tears down the beauty, albeit artificial, of the countryside to grow sugar beet that no one needs. And the tragedy of sugar production is but a repertory-company version of the grander tragedy being acted out in the Amazon basin.

Politics and law are the only tools that can be used to redress these assaults on decent living. So the greens turn political. Alas they also turn socialist, despite the warnings from the Soviet Union and military dictatorships alike that state control does not lead to the preservation of land or biological diversity. But green thinking is inside politics to stay and the greens may yet be the law-givers. ☐

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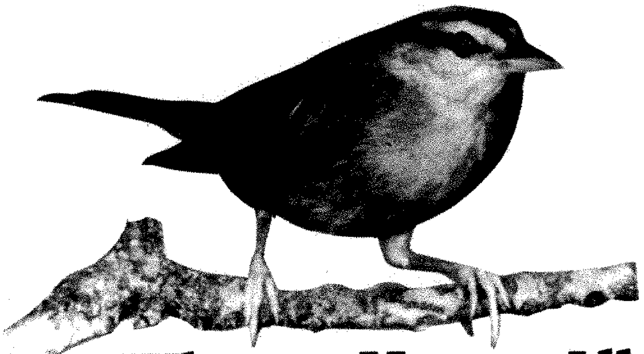

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Warming up the Cold War

Daniel S. Greenberg

Techno-Diplomacy: US–Soviet Confrontations in Science and Technology. By Glenn E. Schweitzer. *Plenum: 1989. Pp.320. $22.95.*

EARLY in the Cold War, Glenn Schweitzer went to Moscow as the scientific officer at the US embassy. Ever since then he has been deeply involved in one of the most perplexing and disputed aspects of the rivalry between the superpowers — their relations in science and technology, which have been maintained to some degree even during the most uneasy periods of the past 30 years.

To the Reagan administration, visiting Soviet scientists were spies, and research in the Soviet Union was too backward to warrant the interest of US scientists. The Reaganites also rejected as infantile the hope that contact with science in the United States would soften Soviet ideology and promote reforms in harmony with Western interests. Schweitzer, who is now staff director for Soviet and Eastern European Affairs at the National Academy of Sciences, makes the contrary case. The issue of 'warmer' or 'cooler' research relations has at present been rendered moot by the glow of *glasnost* but, as Schweitzer points out, the pendulum has swung many times in the post-war years, and may yet swing again. In brief, he makes three points. First, that scientific exchanges are a poor way of collecting strategically valuable research data, and therefore should not be curtailed because of fears of espionage; second, that although Soviet science and technology lag in many fields, other areas are so advanced that US self-interest dictates collaboration with Soviet researchers; and third, that Soviet scientific and technical contacts have helped "blunt the edge of the US–USSR military confrontation", to which, intriguingly, is added the remark that "in Washington, their importance as a rudder of political stability in the rough turbulence of US–Soviet relations has yet to be adequately recognized".

The book contains an odd mixture of officialese and platitudes ("Capabilities in science and engineering spring from a nation's educational system") and fascinating, but often all-too-brief accounts of Schweitzer's personal experience of Soviet science, technology and higher education. For instance, referring to the academic process of thesis defence, he writes: "I have attended some of these sessions; sometimes they are filled with scientific controversy, while on other occasions they seem almost *pro forma* in satisfying an academic requirement". And that's it. Elsewhere, he expresses himself forcefully as when he assails one of the ideological hatchet men of the Reagan era, Richard Perle, for his opposition to Soviet–US scientific relations.

There is no indulgence in romanticism about the brotherhood of science to be found here: for example, "Tourism sometimes becomes a principal motivation for American scientists to participate in exchanges. . . . The ballet and the monastery visits can set the timetable". Likewise, a trip to the West is coveted by Soviet scientists because it gives them the opportunity for a shopping spree. Nor does Schweitzer dispute the contention that the Soviets prey upon Western science and technology to redress their own deficiencies in research. He insists, however, that although Western technology has been useful in some instances, it is not the backbone of the Soviet Union's formidable military power. Furthermore, he points out that technologies that originate in the civilian sector — semiconductors, for example — are increasingly vital to military systems, and are increasingly available on world markets.

Throughout, the weight of the argument is that the strategic risks of scientific amity have been exaggerated while the benefits, scientific and political, have been obscured by right-wing misrepresentations. Along the way, Schweitzer challenges the conventional grumping about Soviet visits to US research facilities, pointing out that exchanges are not an easy way of forging ahead in science. He was formerly director of a laboratory of the Environmental Protection Agency, and notes that he "led many attempts to transfer scientific techniques from our institution to others. . . . We had very advanced techniques, the other laboratories needed them to comply with environmental regulations, and we were eager to facilitate the technology transfer". Nonetheless, bureaucratic inertia and differences in equipment and training requirements meant the process was a time-consuming and difficult one.

As I have mentioned, Schweitzer credits Soviet–US scientific relations with an important role in getting the two countries safely through the Cold War. He suggests that the Soviet Union's scientific window on the West contributed to the country's astonishing ideological turnabout by vividly showing up that country's scientific and technological backwardness. And, on behalf of US interests, he pleads for even closer ties amid the turbulence and uncertainties that currently afflict the Soviet Union.

"Let us hope", he writes, "that our two countries are rapidly emerging from the adolescence of scientific nationalism . . . . Most importantly, American scientists have known and respected for many years Soviet scientific colleagues who are now members of Gorbachev's brain trust and who fully appreciate the need to redirect the Soviet scientific enterprise toward peaceful purposes. The likelihood of their doing so depends to a large extent on the US response to the new opportunities for relaxation of military tensions and for scientific cooperation".

This, he concludes, "is the challenge of techno-diplomacy". □

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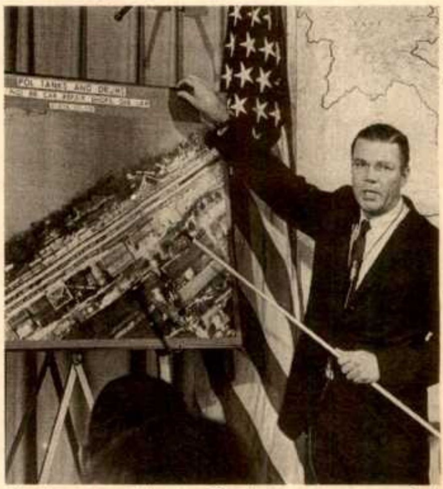
Risky business

Lawrence Freedman

Defending Deterrence: Managing the ABM Treaty Regime into the 21st Century. Edited by Antonia H. Chayes and Paul Doty. *Pergamon–Brassey's, McLean, Virginia: 1989. Pp.286. $32, £20.*

THE 1972 Anti-Ballistic Missile (ABM) Treaty remains one of the most solid achievements of arms control. It was a product of the US–Soviet detente of the early 1970s which came to be associated, eventually, to his embarrassment, with the name of Henry Kissinger. The United States was suffering from the trauma of the Vietnam war and the Soviet Union appeared to be ascendant. Kissinger's hope was to declare a stalemate, create a Soviet stake in the *status quo*, and so prevent a more fundamental challenge to Western interests.



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McNamara giving a Pentagon press conference during the Vietnam war, 1960.

The initiative which eventually led to the treaty can be traced back to the ideas associated with Robert McNamara, US Secretary of Defense for much of the 1960s. While Kissinger understood stability largely in terms of a political arrangement with the Soviet Union, of which an arms-control treaty was an important component, McNamara saw it more as a

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military arrangement.

During his tenure in office, McNamara had stressed the need for a stable military balance. By this he meant that in a crisis neither side would have any incentive to strike first with nuclear weapons in the hope of denying the opponent a prospect of retaliation. The concept was of mutual assured destruction. Stability was a consequence of a shared vulnerability — when both superpowers might be consumed in a conflagration, neither side would light the touch paper.

Anything that could reduce vulnerability might reduce stability. This included defensive weapons. A 'shield' on its own might seem innocuous — the most moral form of armed force — but it could tilt the balance in a contest between two sides hitherto armed only with swords. During the 1960s the Soviet Union, unconvinced by this notion and anxious to build barriers against all possible attacks against its territory, constructed a first-generation anti-ballistic missile system around Moscow. McNamara did not want to imitate the system because he believed, correctly, that new offensive methods, and in particular multiple warheads, could overwhelm any defensive missiles.

Nonetheless, as he was leaving office he was obliged to authorize the construction of a US ABM system, ostensibly designed to cope with the first Chinese missiles. In 1969 the Nixon administration made this more explicitly anti-Soviet, but faced difficulty in gaining public, scientific and congressional support. The system become dispensable and was sacrificed to achieve the 1972 treaty.

Just over ten years after the treaty came into force it was challenged by the Strategic Defense Initiative (SDI), set in motion by President Reagan in 1983. Reagan and his supporters abhorred the idea of mutual vulnerability. It would be better, the president insisted, to "save lives" rather than "avenge" them. He hoped to find means of destroying offensive missiles during the first stages of their flight paths, from boost phase to the re-entry of the nuclear charges into the atmosphere. From early on it was pointed out that such an effort was in direct contradiction to the ABM Treaty. The treaty prohibited not only the deployment, but also the development and testing of all systems other than fixed, land-based interceptor missiles and their associated radars of the sort that had been in place in 1972 and that could not be modernized, albeit within very strict numerical limits (initially 200 and then later 100 interceptors for each side).

Once the president's will was known the initial reaction from the Pentagon was that, should the research appear promising, the ABM Treaty could be dispensed with if necessary. However, instead of mounting such a direct assault on an arms control treaty, at a time when the president was claiming to favour arms control in principle, a more indirect approach was adopted, exploring what might be permitted on generous interpretations of the treaty's provisions. The treaty only covered 'strategic' missiles; might it be possible to develop a system to deal with 'tactical' missiles in Europe? There was overlap between anti-satellite and anti-missile weapons; might the former be used as a cover for the development of the latter? The treaty limited the testing of 'components', but what might be done with sub-components? There was a complicated provision concerning the testing of systems based on 'new physical principles'; despite uncomplicated and apparently unambiguous language to the contrary, could this be construed as allowing the development and testing of anything involving new technologies?

This assault on the treaty was complicated by the Soviet response to the SDI. Instead of simply saying that if it went beyond research it would be prohibited, Soviet spokesmen insisted that even research was not allowed. Later they proposed an extended mutual commitment to staying within the treaty's terms, but by this time the main issue had become the Reagan administration's broad interpretation of the terms. The Soviet position was not helped by doubts over its own compliance. Matters became more complicated with proposals to get out of the impasse by considering agreed experiments that could be undertaken without undermining the treaty completely.

Those who prefer the known strengths of the treaty to the doubtful promise of SDI have thus been forced to pit technicality against legality. The number of people truly competent to do this are few and most of them have contributed to the book under review. All of the main areas of controversy are addressed. The conclusion, not surprising from the book's title, is that the purpose and constraints of the treaty are far clearer than those who have sought to undermine them would have us think. I do not believe that the opposing view could have assembled anything approaching the same weight of argument and expertise, even though it notionally remains that of the Bush administration.

Defending Deterrence is not an easy read, and it would have been refreshing to have had a more critical look at the concept of deterrence which is being defended. But the book is comprehensive, authoritative and lucid. Should negotiations on the future of the treaty gather pace during the coming months it will be hard to find a more useful work of reference; if the two governments decide that it is all too difficult, then Chayes, Doty and their contributors will help explain why. □

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Rules of conduct

John A. Campbell

Benefits and Risks of Knowledge-Based Systems. Report of a Working Party of the Council for Science and Society, chairwoman Margaret Boden. *Oxford University Press: 1989. Pp. 76. Pbk £6.95, $14.95.*

ARTIFICIAL intelligence (AI) is in the news less often now than it was in the early and middle 1980s. At present there is nothing quite like the world-wide interest in the Japanese 'Fifth Generation' plan or in the novelty and first commercial successes of expert systems that produced all the previous attention. But AI research operates rather like a volcano: there is constant activity underground, which becomes visible only during eruptions. It is likely that neural nets will cause the next eruption, and in the not-too-distant future.

Among AI researchers there is the saying "AI exports its successes". What this usually means is that the wider public that makes use of the successes no longer sees them as part of AI (the languages LISP and Prolog are examples). Now expert systems seem to be going in the same direction, like lava that is cooling down into easily-handled pieces of stone.

In some quarters, this comfortable trend towards cooling or domestication is even seen as not being fast enough. In the ESPRIT programme, which is an EEC response to the Fifth Generation plan, calls for proposals for research and development projects regularly contain subject-headings such as "metrication and validation of knowledge-based systems". The implication is that computer programs built around knowledge (essentially the rule-based knowledge that is the distinguishing feature of a typical expert system) should eventually be metricated, validated and domesticated with no more trouble than for book-keeping or ticket-issuing programs.

To do the writers of the ESPRIT material justice, they appreciate that they are expressing a hope rather than a certainty. It is unfortunate that this appreciation is not more widespread, especially among people who use or rely on expert-systems technology. The book reviewed here illustrates some of the issues by highlighting the present limitations of the technology, and makes suggestions about how to deal with them. It is welcome because the suggestions are modest and reasonable — for example, that the systems should be treated not as oracles but as assistants to people performing tasks that require specialized knowledge, and that it should be possible for a user to examine the knowledge represented in them. But there is a risk that the book's modesty and low profile will mean that its arguments go unnoticed. Here is a rare case of a book's packaging being too sober.

The special character of knowledge-based systems (KBS) follows from the fact that knowledge is multi-faceted, open-ended, ambiguous and even scruffy, while the functions and specification of (say) book-keeping programs are not. Admittedly there are areas in which the relevant knowledge is well-behaved and can be given the book-keeping treatment, as in some of the most commercially successful expert systems, but these examples do not mean that all conceivable or desired KBS must have the same properties. One possible criticism of the book is that, by using the terms 'expert system' and 'knowledge-based system' almost interchangeably, it misses a chance to sharpen this point.

Clearly a KBS is founded on knowledge, in whatever representations are available to hold it. An expert system obviously has something to do with expertise, but its original meaning (most of which still survives, particularly in the United States), was tied quite tightly to one single representation for knowledge: *rules*. The most interesting KBS may use rules, but other representations such as frames (collections of information that belong together in some particular context) and inheritance nets (hierarchical structures in which specific items inherit properties from more general items, of which they are particular instances) are used at the same time. This reflects the actual organization of expert knowledge much better, and extends the range of AI applications beyond what was available in the early 1980s. But it also emphasizes the difficulty of predicting all the outcomes and pitfalls when some particular body of knowledge that goes beyond mere rules is represented and used inside a KBS. In this respect, the book gives a fair summary of the issues that deserve to be confronted, even though simple rules are the only examples of knowledge that it uses. To get the most out of the case that it makes, the reader should repeat regularly: "There is more to knowledge than rules".

The fundamental reason for the mixture of cautions and actions that the authors' collective for this book recommends is that a KBS is unlikely to use the same chain of reasoning that a human would use to solve the same problem. The methods of inference in a KBS are local approximations to our own reasoning for parts of problems — sometimes optimistic approximations (for example, the psychological evidence that we do any reasoning with the help of logic as a mathematical logician would describe it is very thin). But there is no general AI method that imitates our perception and treatment of a reasoning problem as a whole. This gives force to the recommendation that a KBS should be used as a human's assistant and not (unless provably domesticated) trusted as an autonomous agent, especially where human life and safety are concerned. An assistant who suggests initiatives should always be capable of explaining them in terms that a human senior partner can understand and accept. This concentrates attention on the ability of a typical KBS to explain its actions, and here there is still plenty of room for improvement. Explanation, or lack of it, is also likely to be the main weakness of neural-net systems, despite their strengths in other respects. Therefore, the book's relevance will not be changed by the arrival of neural nets on the commercial scene, if this indeed causes the next eruption of publicity for AI.

Relevance and reasonableness alone are not enough to make a political impression, alas. Subversion of the established (commercial) order is a better way in 1989. If the author's subversive recommendation that a "human or group of humans ... should take moral and/or legal responsibility for the human consequences of any malfunction" of any KBS does not stir up some debate, then nothing will. ☐

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Kume, H Regulation of Ca^{2+}-dependent K^+ channel activity in tracheal myocytes by phosphorylation (*with* Takai, A, Tokuno, H, Tomita, T) *Letter to Nature*, **341**, 152
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Lundin, R First measurements of the ionospheric plasma escape from Mars (*with* Zakharov A Pellinen R Borg H Hultqvist B Pissarenko N Dubinin E M Barabash S W Liede I Koskinen H) *Letter to Nature* **341**, 609
Luzzatto, L Protein structure One enzyme from two genes? *News and views* **341**, 286
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McVey, D Phosphorylation of large tumour antigen by cdc2 stimulates SV40 DNA replication (*with* Brizuela L Mohr I Marshak D R Gluzman Y Beach D) *Article* **341**, 503
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Murchie, A I H Fluorescence energy transfer shows that the four way DNA junction is a right handed cross of antiparallel molecules (*with* Clegg R M Kitzing E von Duckett D R Diekmann S Lilley D M J) *Letter to Nature* **341**, 763
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ky, M B Crabtree, G W, Dunlap, B D, Griessen, R P, Maekawa S, Osipyan, Yu A, Ott, H R, Tanaka, S *editors*) **341**, 368
Journal review of Journal of Superconductivity (Gubser, D U, Wolf, S A *editors*) **341**, 368
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Siekierka, J J A cytosolic binding protein for the immunosuppressant FK506 has peptidyl-prolyl isomerase activity but is distinct from cyclophilin (*with* Hung, S H Y, Poe, M, Lin, C S, Sigal, N H) *Letter to Nature,* **341**, 755
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QUALIFICATIONS include a Ph D, and at least seven-year experience in one of the three above-described specialized areas, and an excellent record of scientific achievement Successful candidates will have a chance to be promoted to the Division Chief, who is expected to have supervisory responsibilities directing junior staff

SALARY will be commensurate with experience and qualifications We offer house accommodation, and benefits package together with the opportunity to work in our newly constructed superb facilities equipped with modern technologies

A letter of application, C V, list of publications, three professional referees, and your portrait should be sent by the end of December 1989 to the following address

Yutaka Tomita, Managing Director,
Technical R & D Division,
Terumo Corporation,
44-1, 2-chome, Hatagaya,
Shibuya-ku, Tokyo 151, Japan
Phone 03(374)8020, Fax 03(374)7493

Applications will be reviewed continuously until suitable candidates are hired Promising candidates will be contacted for further discussions on the terms of employment contract

(W6595)A

TERUMO CORPORATION

Institut fur Genbiologische Forschung Berlin GmbH

POSTDOCTORAL POSITION

for a Biochemist/Molekular Biologist

A postdoctoral research fellow is required to work on a joint programme with the **Schering AG Agrochemical Research** involving the characterization of plant proteinase inhibitors and their genes The Position is available for 2–3 years

Applicants should have experience with modern methods of protein isolation and analysis and a strong background in molecular biology techniques

Please submit a full c v and the names and addresses of two referees to **Prof Dr Lothar Willmitzer, Institut fur Genbiologische Forschung Berlin GmbH, Ihnestrasse 63, 1000 Berlin 33, Fed Rep Germany**

(W6714)A

AGRICULTURAL AND FOOD RESEARCH COUNCIL (AFRC)

HEAD OF AFRC INTERNATIONAL OFFICE

SWINDON **SALARY £17,360 – £22,606**

plus performance related increments to £26,958 – pay award pending

Already providing research to solve some of the UK's most pressing agricultural and food concerns, the AFRC is committed to developing international research cooperation and to increasing its contribution to the needs of the world's developing nations

COULD YOU IMPLEMENT AND DRIVE THE COUNCIL'S INTERNATIONAL POLICY?

- Improving awareness of the AFRC as a major research resource among international agencies and industrial customers in developed and developing countries around the world
- coordinating the marketing of AFRC's expertise and providing a channel of information on international research opportunities
- developing an early intelligence system for overseas research funding opportunities
- contributing to research policy formulation

Encompassing the many demanding aspects of marketing, technical liaison, presentation, contract negotiation, professional collaboration, targeted fund raising and staff management exercised in a research environment, this challenging position requires a diverse range of skills

If you are an innovative thinker, ideally with a background of both international scientific and administrative experience, the capacity to comprehend and interpret scientific information over a wide range of disciplines and the mobility to travel, please contact us Benefits include non-contributory pension scheme, generous annual leave and flexible working hours

Please apply in writing for an application form and further particulars to Manager, Central Office, AFRC, Wiltshire Court, Farnsby Street, Swindon, SN1 5AT

The closing date for receipt of applications is 1st December 1989

The AFRC is an Equal Opportunities Employer (2113)A

AFRC

POSTDOCTORAL FELLOW or RESEARCH ASSISTANT

A three-year appointment is immediately available for an organic chemist with an interest in biology, to work in the new field of catalytic antibodies (abzymes) These molecules are thought to have great potential in stereospecific catalysts The post is funded by Shell Research Ltd, Sittingbourne and will be held jointly in the Departments of Chemistry and Biology (Cancer Research Unit) at the University of York The successful candidate will be expected to show independence of thought and an ability to bridge biology and chemistry If the successful candidate is employed as a research assistant, they would be expected to register for a higher degree Only candidates with at least a 2 i need apply

Salary within the Research and Analogous scales (£9,816 to £12,381 – grade IB, or £10,458 to £16,665 – grade IA)

Informal enquiries to Dr Colin Garner on 0904-432900

Three copies of applications, with full curriculum vitae and naming two referees, should be sent by 5 December 1989 to the Personnel Office, University of York, Heslington, York YO1 5DD Further particulars are available. Please quote advertisement reference number 6/6280 (2077)A

UNIVERSITY OF YORK

UNIVERSITY OF NOTTINGHAM MEDICAL SCHOOL

Depts of Biochemistry and Genetics

POSTDOCTORAL RESEARCH ASSISTANT GRADE 1A

Applications are invited for a post-doctoral research assistant to work with Dr P Jones and Dr J Brookfield on a project funded by the Medical Research Council to study the role of the protein vinculin in actin reorganisation and cell adhesion during Drosophila embryogenesis The appointment is for 3 years on the 1A scale and is available immediately Experience of Drosophila genetics and/or molecular biology techniques would be an advantage, but not essential

Applications with the names and addresses of two referees should be sent to **Dr P Jones, Dept of Biochemistry, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH,** from whom further details may be obtained (tel 0602-709365) (2044)A

UCL

UNIVERSITY COLLEGE LONDON

Department of Biology

TWO APPOINTMENTS IN PLANT PATHOLOGY/MYCOLOGY

1 A post-doctoral research assistant is required immediately to study the morphological and biochemical basis of the resistance of wheat to the fungal pathogen *Fusarium graminearum* Work on the project will be in collaboration with an Agricultural Station in Uruguay and CIMMYT, Mexico Candidates should have good practical experience of general biochemical techniques

The appointment, which is funded by the Overseas Development Administation, is for three years and will be on the RA 1A scale The initial salary will be determined by age and experience and will be up to a maximum of £14,169 + £1,650 London Allowance

2 A post-graduate, who may register for the Ph D degree, is required to investigate the occurrence of moulds and mycotoxins in oilseed rape at harvest, during storage and following conversion into cake Work on the project will be in collaboration with the Overseas Development and Natural Resources Institute and laboratories in Italy and Pakistan Candidates should have some knowledge of the culture and identification of fungi and the quantitative analysis of biologically active compounds

The appointment, which is for three years, is funded by the EEC The successful candidate will be renumerated at the standard SERC studentship rate (currently £4690 + travel allowance)

Further particulars may be obtained from **Dr R N Strange, Department of Biology, Darwin Building, University College London, Gower Street, London WC1E 6BT** to whom applications, including cv and the names and addresses of two referees should be sent (1921)A

FACULTY POSITION IN BIOPHYSICS AT HAVERFORD COLLEGE

The Department of Physics invites applications for a tenure-line assistant professorship in biophysics Applicants must have a Ph D and be strongly motivated toward creative research and undergraduate teaching The successful candidate will assist in developing a new interdisciplinary biophysics program Substantial start up funds for research are available

Haverford is a liberal arts college outside Philadelphia with an international reputation for strong research and instruction in the sciences Please send curriculum vitae, list of publications, a statement of research and teaching interests, and at least three letters of recommendation by 15 January 1990 to **Professor Lyle Roelofs, Department of Physics, Haverford College, Haverford, PA 19041, USA** *An Equal Opportunity/Affirmative Action Employer* Women and minority candidates are particularly encouraged to apply (NW4573)A

Institute of Terrestrial Ecology

INTERNATIONAL PROGRAMME OFFICER

Tropical Soil Biology and Fertility Programme

A Programme Officer is sought to act as co-ordinator of an international programme of research into the biological bases of tropical soil fertility The Programme is sponsored by the International Union of Biological Sciences and UNESCO, but the post is with the Institute of Terrestrial Ecology, Edinburgh

The appointee will develop the conceptual basis of the Tropical Soil Biology and Fertility Programme (TSBF) through literature review, integration of data from TSBF sites and participation in model development He/she will help to organise and co-ordinate activities between Programme Centres throughout the tropics and with the African network The successful candidate will be responsible to Director TSBF

Applicants should have a first or upper second class honours degree with at least two years' relevant post-graduate experience Tropical experience and expertise in modelling will be an asset

The appointment will be for a period of three years, salary will be in the range of **£10,678** to **£14,909** pa or **£13,254** to **£18,864** pa depending upon experience and qualifications, plus relevant foreign allowances The successful candidate will be stationed at either - Nairobi, Kenya with Dr J Ingram, Co-ordinator of African Network, TSBF or Ibadan, Nigeria with Professor M J Swift, Director TSBF Terms include generous holidays and a non-contributory pension scheme

The Institute of Terrestrial Ecology is a component body of the Natural Environment Research Council

Further details and an application form are available from David Tyers, Institute of Terrestrial Ecology, Edinburgh Research Station, Bush Estate, Penicuik, Midlothian, EH26 0QB Tel 031-445-4343 Fax 031-445-3943 Telex 75259 Closing date for receipt of completed application forms is 30th November 1989

The Natural Environment Research Council is an Equal Opportunities Employer

**Natural
Environment
Research
Council**

(2117)A

**MRC Clinical Research Centre
Watford Road
Harrow, HA1 3UJ**

crc

MOLECULAR BIOLOGIST

This Post-Doctoral position, which is available initially for 2 years, is a joint project between the Haemostasis Research Group (CRC), MRC Collaborative Centre (Mill Hill) and Biotechnology Interface Ltd on production of plasma proteins by transgenic technology The successful applicant will have experience in molecular biology and a strong desire to acquire the techniques of gene transfer to laboratory animals Further details may be obtained from Dr J McVey 01 869 3173 Ref ET/BI

Salary will be at an appropriate point on the salary scales for non-clinical university academic staff

The Institute has a staff of 500 and is linked with a district general hospital which serves a local community of some 215,000 people

There is easy access to British Rail and London Underground (Metropolitan and Bakerloo lines) and buses Staff may obtain interest free season ticket loans

Facilities include subsidised canteen, occupational health, sports and social club, bank and shops on site

Application forms may be obtained from the Personnel Dept, tel 01 869 3431

Closing date for completed applications is 8th December 1989

The Medical Research Council is an Equal Opportunity Employer (2096)A

THE UNIVERSITY OF
MELBOURNE
Australia

CHAIR OF ZOOLOGY

The University of Melbourne
vites applications for a new C
of Zoology The existing Cha
the department is filled by
fessor G D Campbell

The appointee will be a di
guished Zoologist and be prep
to give academic leadership in
Department and in biolo
sciences within the Univer
The Department of Zoology
well funded research group
comparative, cardiovascular,
piratory and automatic ph
logy, evolutionary biology, ne
ethology and behaviour,
marine ecology

It is anticipated that the app
tee will serve as Head of
Department for a period of u
five years

Salary A$63,919 per ann
The University is currently ex
ining the question of salary l
ings and responsibility allowan

Further information about
position, application proced
superannuation, travel and
moval expenses, housing as
ance and conditions of app
ment is available from the R
trar, or from Appointm
(37165), Association of Comn
wealth Universities, 36 Gor
Square, London WC1H 0PF
correspondence (marked "C
dential") should be addresse
the Registrar, The Universit
Melbourne, Parkville, Vict
3052, Australia (Tel +61 3
7528 Facsimile 344 6897)

Applications close on 23 F
ruary, 1990

The Council reserves to i
the right to make no appointm
or to fill the Chair by invitatio
any stage

An equal opportunity
ployer (W6718)

UN GRAND LABORATOIRE PHARMACEUTIQUE
FRANCAIS (PARIS) RECRUTE

DEUX PHARMACOLOGUES CARDIOVASCULAIRE ET CELLULAIRE

Biochimistes, véterinaires, ingénieurs agronomes, pharmaciens ou universitaires scientifiques, agés d'environ 35 ans, les candidats ont acquis une experience de la fonction dans un laboratoire de recherche public ou dans l'industrie pharmaceutique Ils maîtrisent les techniques d'analyse in vitro et la radioactivité Ils parlent l'anglais et utilisent l'outil informatique

Tous les candidats seront jugés sur leur capacité a developper rapidement des produits

REF 504

UN GALENISTE

Agé d'environ 30 ans, pharmacien, chimiste, ingénieur généraliste (option chimie macromoléculaire), le candidat idéal a une experience de 5 ans en Galenique acquise dans une société utilisant les biomatériaux (collagene, polymères de synthèse) Il maîtrise l'anglais et utilise l'informatique Il a une bonne aptitude au développement

REF 503

Apply box no W6731A c/o 4 Little Essex Street, London WC2R 3LF

(W6731)A

COLUMBIA UNIVERSITY
Center for
Radiological Research

taff Associate Position

iologist experienced in adiation biology, cell culure, transformation *in vitro* nd molecular biology to arry out research in these reas

nterested individuals should rite to **Dr C Borek, Center r Radiological Research, olumbia University, 630 Vest 168th Street, New ork, N Y 10032, USA**

olumbia University is an qual opportunity/affirmave action employer

(NW4574)A

OSTDOCTORAL POSITIONS

vailable to study gene specific epair of oxidative damage to DNA f mammalian cells and purification nd cloning of DNA glycosylases ffecting repair of such damage ecent PhD with experience in rotein chemistry, molecular biolgy or DNA chemistry is preferred alary and benefits are competive Please send CV and names of eferences to **G W Teebor, M D, ept of Pathology, NYU School of ledicine, MSB 605, 550 First Ave, IYC, NY 10016** (NW4569)A

★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★

THE COMMISSION OF THE EUROPEAN COMMUNITIES

is organizing a selection procedure in order to establish a reserve list for the recruitment of staff in grade B/2 for its research programmes (Recruitment will be on the basis of temporary contracts)

NUCLEAR CHEMISTRY

Chief laboratory technician

(grade B/2 – COM/R/B/11)

Duties to carry out complex duties of analysis in the frame of research programmes in the Mass Spectrometry Laboratory

Qualifications/Experiences ☐ at least 10 years experience in high precision isotope analysis and in maintenance of FINNIGAN-MAT instruments, ☐ knowledge required in electronics and computer programming, ☐ experience in supervision of the daily running of the lab

GENERAL CONDITIONS

Nationality candidates must be a national of one of the Member States
Place of work Joint Research Center (Institute for Transuranium Elements) - KARLSRUHE
Age limit 45 years maximum
Knowledge of languages knowledge of one Community language (Danish, Dutch, English, French, German, Greek, Italian, Portuguese and Spanish) and a satisfactory knowledge of a second Community language
Closing date requests for application forms must be made in writing not later than 15/12/1989 (postmark) to the following address

C E C , Secretariat for Selection Committees Research,
SDME R2/54, 8 Square de Meeûs, B-1049 Brussels, Belgium
(telephone 02/235 56 60 - telefax 02/236 30 25)

Application forms duly completed and signed, must reach the above address not later than 15/01/1990

(W6710)A

★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★★

UNIVERSITY OF BRITISH COLUMBIA
Department of Microbiology

Molecular Immunologist

The Department of Microbiology, University of British Columbia invites applications from molecular immunologists for an assistant professor level, tenure track position The individual we seek will have a Ph D, postdoctoral experience, a strong research record and show potential for developing an internationally-recognized research program which will complement ongoing programs in immunology, virology and bacterial pathogenesis Salary will be commensurate with qualifications and experience The successful candidate will be expected to participate in the Department's large and expanding Ph D progarm In accordance with Canadian immigration requirements priority will be given to Canadian citizens and permanent residents of Canada Closing date for applications is February 28, 1990 Position will be available July 1, 1990 The position is subject to final budgetary approval

Please send curriculum vitae, a summary of research interests and the names of three referees to **The Head Department of Microbiology, University of British Columbia, #300 6174 University Boulevard, Vancouver, B C V6T 1W5**

The University of British Columbia is committed to the Federal Government's employment equity program and encourages applications from all qualified individuals (NW4517)A

ASSISTANT/ASSOCIATE PROFESSOR DEPARTMENT OF CELL BIOLOGY

The Department of Cell Biology is accepting applicaitons for a tenure-track position at the Assistant or Associate Professor level Applicant must be qualified to teach gross anatomy to medical students Preference will be given to candidates with teaching experience in gross anatomy in addition to research experience Research interests should complement existing research strengths of the department developmental biology, reproductive biology, neurobiology, growth factors

Send curriculum vitae, description of research interests, and the names of three references to **Paula C Hoos, Ph D , Chairperson of Search Committee, Department of Cell Biology, Vanderbilt School of Medicine, Vanderbilt University, Nashville, Tn 37232–2175**

Vanderbilt University Medical Center is an Equal Opportunity, Affirmative Action Employer (NW4565)A

PHOTOSYNTHESIS RESEARCH GROUP

UCL

Post-doctoral Research Associate

An SERC supported post-doctoral position is available for two years from 1st January 1990 for work on the structure and function of photosystem I

The research programme will involve work on the isolation and purification of photosystem I to determine the role of the smaller polypeptides in electron transport and the application of advanced electron paramagnetic resonance spectroscopy to investigate the structure, and electron transfer mechanisms, in the electron acceptor complex

A background in Biochemistry, Biophysics or Physical Chemistry would be appropriate Training will be available for novel techniques

Salary on the RA 1A scale £10,458-£16,665 plus £1,650 London Allowance

Applications including c v and names of 2 referees should be sent to **Prof M C W Evans, Dept of Biology, Darwin Building, University College London, Gower St , London WC1E 6BT, 01-380 7312,** from whom further details may be obtained Equal Opportunities Employer (2107)A

University of Nottingham
Department of Botany

POST-DOCTORAL RESEARCH ASSISTANT IN PLANT PATHOLOGY

Applications are invited for an AFRC funded research post to work on the genetic control of host species specificy in the downy mildew pathogen *Peronospora parasitica* The 3 year project will utilize rapid-cycling *Brassica* lines and tissue culture approaches to produce hybrids between host genotypes which differ in response to defined pathotypes of the fungus The sexual cycle of the fungus will be used to generate progeny in which the segregation of host range genes will be analysed The project will be based in the Plant Pathology laboratory at Nottingham but will also involve collaborative work in tissue culture and with the Institute of Horticultural Research

Applicants should have appropriate research experience in plant pathology, plant breeding, tissue culture, or genetics Knowledge of molecular techniques would be an advantage The appointment will be on the 1A scale £10,458 to £13,527 Applications should be sent by 1st December to Dr J A Lucas, Department of Botany, University Park, Nottingham NG7 2RD, from whom further details can be obtained (2087)A

nature

The international journal of science with 500,000 readers throughout the world seeks

A TALENTED BIOLOGIST

Essential qualifications

- A sound education in some field of biology
- A critical and lively interest in science in general
- A sense of literacy

Research experience desirable

Applications are invited from people seeking a career in the communication of science The successful applicant will join the team responsible for assessing and editing biology manuscripts, either in Washington or in London

There will be opportunities for contributing to *Nature* in other ways Research experience in neurobiology or immunology would be particularly welcome, but those with other interests should not be deterred from applying

US applicants should send their *curriculum vitae*, with a detailed account of their education, research experience and literary skills, to the

Editor, *Nature*,
1137 National Press Building,
Washington, DC 20045

by 20 November and be prepared to be interviewed in Washington shortly thereafter

Applications from elsewhere should be sent to the

Editor, *Nature*,
4 Little Essex Street, London WC2R 3LF

by 27 November (1997)A

MOLECULAR PARASITOLOGY

The **Case Western Reserve University School of Medicine** is seeking applications for a faculty position in the **Department of Molecular Biology and Microbiology** Individuals using modern molecular biological methods to study gene expression in a parasitic organism are encouraged to apply While the prospective rank of this appointment is open, preference will be given to individuals with an established, independent research program CWRU offers a large and interactive research community in Molecular Biology which includes several investigators whose major research interest is in the molecular aspects of parasite gene regulation

Curriculum Vitae should be submitted by February 1, 1990 to

Dr T W Nilsen
Search Committee for Molecular Parasitology
Department of Molecular Biology & Microbiology
Case Western Reserve University, School of Medicine
Cleveland, Ohio 44106

CWRU is an Equal Opportunity/Affirmative Action Employer

(NW4570)A

PUBLIC HEALTH LABORATORY SERVICE CENTRAL PUBLIC HEALTH LABORATORY NATIONAL COLLECTION OF TYPE CULTURES BASIC GRADE MICROBIOLOGIST

(Mycoplasma Reference Facility)

Graduate with an honours degree with a major microbiological component needed to assist in routine and research laboratory work with mycoplasmas Training in specialized techniques will be given

Salary scale £8,135 - £11,830 per annum (inclusive) Major pay review pending

Further information may be obtained from Dr R H Leach (tel 01-200 4400, ext 3756) Closing date 1 December 1989 Please quote ref NCTC/1

Application from and job description from Personnel Department, Central Public Health Laboratory, Colindale Avenue, London NW9 5HT Telephone No 01-200 4400, ext 3890

(2070)A

UNIVERSITY OF CAMBRIDGE DEPARTMENT OF ZOOLOGY

UNIVERSITY LECTURESHIP

Applications are invited for a Lectureship in the Department of Zoology Applicants should have a research interest in the field of Cell Biology, preferably in the cytoskeleton or a related area The Department has a strong interest in many aspects of biological science, including cell and molecular biology, developmental biology and neurobiology

The appointment to the lectureship will be for 3 years in the first instance, with the possibility of reappointment to the retiring age The scale of stipends for a University Lecturer not ordinarily resident in College is £14,169 pa, rising by eleven annual increments to £21,850

Please ask for further information on the post and the Department from **John Andrews, Secretary to the Appointments Committee for the Faculty of Biology 'A', Department of Zoology, Downing Street, Cambridge, CB2 3EJ Tel 0223 336621 (International + 44 223 336676)** Applications (twelve copies) should include a *curriculum vitae*, list of publications, statement of intended research and the names of not more than three referees The closing date has been extended to Friday 22 December 1989 (2081)A

The University of Manitoba
Faculty of Science

VIROLOGIST/MOLECULAR BIOLOGIST

The Department of Medical Microbiology, University of Manitoba, invites applications for a tenure-track position at the rank of Assistant or Associate Professor, beginning July 1, 1990

Applicants should hold a Ph D or M D degree or both, and should have research experience and a strong publication record in Virology/Molecular Biology of microbial pathogens Other areas of research in cell biology/microbiology will also be considered The successful candidate will be expected to establish an active research program and participate in undergraduate medical and graduate teaching programs Salary will be commensurate with experience and qualifications

Both women and men are encouraged to apply In accordance with Canada Immigration, priority will be given to Canadian citizens or permanent residents

Send curriculum vitae, a summary of research interests, and names and addresses of three referees to **Dr Robert Brunham, Professor and Chairman, Department of Medical Microbiology, University of Manitoba, 543-730 William Avenue, Winnipeg, Manitoba, Canada, R3E 0W3** Applications will be received until January 31, 1990 (NW4581)A

DREXEL UNIVERSITY ENDOWED CHAIR ECOLOGY, ENVIRONMENTAL SCIENCE

Nominations and applications are sought for an outstanding scientist to occupy an endowed chair in the College of Science named in honor of L Drew Betz, Drexel alumnus and founder of Betz Laboratories, Inc We seek an individual who will provide a focus of excellence for research, instruction, and interaction with students in areas related to ecology and the environment The Bioscience Department has interests in ecology, environmental science, biochemistry and molecular biology and the University's Institute for Environmental Studies has interests in water and air quality, water supply, and solid waste management Initial screening for the position will begin 15 December 1989 and continue until a suitable candidate is found, or until Aug 31, 1990

Submit nominations, inquiries, curriculum vitae, statement of interests, and names of at least three references to **Dr James R Spotila, Chairman Betz Chair Search Committee Department of Bioscience and Biotechnology Drexel University, Philadelphia, PA 19104**

Drexel University is an Equal Opportunity/Affirmative Action Institution and invites applications from women and minorities (NW4572)A

POSTDOCTORAL TRAINING IN CELL REGULATION

The Department of Biochemistry, St Jude Children's Research Hospital, announces a training program in the molecular and cellular biology of cell growth, differenti-ation and transformation The program emphasizes a multidisciplinary approach that develops skills in biochemistry, molecular genetics and cell biology Fellows benefit from training in energetic, well-funded laboratories within an expanding research institute We offer competitive stipends plus the benefit of living in a grow-ng, economical, sunbelt community Specific research groups participating in the program include

W Y Cheung	Regulatory properties and biological functions of calmodulin, Ca^{2+} regulated enzymes
Cleveland	Growth factor signal transduction and regulation of gene transcription
A Fried	The ubiquitin system and post-translational regulatory pathways
N Ihle	Mechanisms of hematopoietic stem cell growth regulation, differentiation and transformation
S Jackowski	Regulation of phospholipid metabolism and the production of lipid-derived second messengers
C Rock	Cell cycle regulation of membrane biogenesis
E Thomas	Antimicrobial and antitumor mechanisms of leukocytes

Further information on individual research programs can be obtained by writing the Department of Biochemistry Applicants should indicate the research group(s) they are nterested in, submit a brief statement of their research nterests, curriculum vitae, reprints, and names of three references to

Postdoctoral Training Program
Department of Biochemistry
St. Jude Children's Research Hospital
332 N. Lauderdale
Memphis, TN 38105

An Equal Opportunity/Affirmative Action Employer

(NW4568)A

UNIVERSITY COLLEGE LONDON
UNIVERSITY OF LONDON
INSTITUTE OF ARCHAEOLOGY
READER/SENIOR LECTURER IN ARCHAEOLOGICAL CONSERVATION AND/OR MATERIALS SCIENCE

Applications or nominations are invited for this senior position in the nstitute of Archaeology The appointee will be expected to provide research leadership in the conservation of archaeological and ethnographic materials and/or in the science of materials used in antiquity and by pre-industrial societies He/she will contribute actively to the further development of conservation within the internationally known Department of Archeological Conservation and Materials Science, and will share in the undergraduate and postgraduate teaching in the Department which provides courses in conservation, ancient materials technology, and museum studies He/she will be expected to take a major role in the planning and implementation of the Institute's projected new Centre for Conservation and Museum Sciences, and to participate in fund-raising activities

The successful applicant will have research experience in an appropriate scientific discipline such as applied chemistry or materials science, preferably with expertise in conservation or in the study of ancient materials Applicants may be from university, museum, governmental or commercial organisations The initial salary will be in the range £23,139 – £27,903 pa inclusive of London weighting

Applications or nominations (one copy suitable for photographic reproduction) giving full details of qualifications and experience, and the names of three referees, should be sent **not later than 31 January 1990 to the Director's Personal Assistant, Institute of Archaeology, University College London, 31-34 Gordon Square, London WC1H 0PY** (FAX Number 01 387 8057) from whom further particulars can be obtained

Equal opportunities employer (2086)A

UNIVERSITY OF NATAL

PIETERMARITZBURG,
SOUTH AFRICA

The University of Natal rejects apartheid It is an equal opportunity, affirmative action University

Faculty of Agriculture

Professor of Crop Science

The South African Sugar Association Chair of Crop Science

Ref P93/89
Closing date 10 January 1990

The Department of Agronomy (which includes the sub-disciplines of Crop Science Soil Science and Agrometeorology), specifically the Crop Science Section provide broad-based curricula designed to equip graduates for careers in research extension and crop production in both commercial and subsistence agriculture sectors and has an active postgraduate school Majors in agrometeorology and soil science are also offered in the combined Department

Applicants should hold a doctoral degree

Chair of Genetics

Ref P91/89
Closing date 10 January 1990

The Department of Genetics is housed in the Faculty of Agriculture and is responsible for teaching and research in Molecular Genetics, Quantitative Genetics and Plant Breeding Students are attracted from both the Faculties of Agriculture and Science

Scientists with teaching and research experience in at least one of the above fields of genetics are invited to apply for the vacant professorship

A doctorate in genetics is essential and applicants must be able to provide academic leadership and should have a proven record of achievement in teaching post-graduate supervision and research

The salaries offered will be determined according to the qualifications and/or experience of the successful applicants

Application forms, details of the salary package, further particulars of the posts and conditions of service are obtainable from the Personnel Section, University of Natal, P O Box 375, Pietermaritzburg 3200, South Africa, telephone (0331) 63320, fax no 27-0331-63497 or the Secretary, South African Universities Office, Second Floor, 16 Charles II Street, London SWIY 4QU, telephone 01-839-3420 or 01-839-4388, fax no 01-839-3480

(W6729)A

Department of Virology University of Freiburg, West-Germany Faculty Position in Molecular Virology (C1)

available for maximum 6 years for Ph D or M D with interest in molecular mechanisms of HIV-induced pathogenesis Postdoctoral experience in recombinant DNA technologies, methods to study protein-nucleic acid interaction and immunochemical techniques is essential The applicant is expected to successfully apply for funding of own projects

Annual salary ranges from 48 000 - to 65 000 - DM Applications with full C V list of publications, statement of research interests and names of two referees should be sent by 31 December to **Dr J Schneider, Abteilung Virologie, Institut fur Medizinische Mikrobiologie und Hygiene, Hermann-Herder-Str 11, D-7800 Freiburg, FRG** (W6728)A

Department of Virology University of Freiburg, West-Germany Faculty Position in Molecular Virology (C1)

available for maximum 6 years for Ph D or M D with interest in molecular mechanisms of HIV-induced pathogenesis Postdoctoral experience in recombinant DNA technologies, methods to study protein-nucleic acid interaction and immunochemical techniques is essential The applicant is expected to successfully apply for funding of own projects

Annual salary ranges from 48 000 - to 65 000 - DM Applications with full C V list of publications, statement of research interests and names of two referees should be sent by 31 December to **Dr J Schneider, Abteilung Virologie, Institut für Medizinische Mikrobiologie und Hygiene, Hermann-Herder-Str 11, D-7800 Freiburg, FRG** (W6728)A

Institute of Terrestrial Ecology

PLANT ECOLOGIST

Monks Wood, Cambridgeshire

Applications are invited for the position of post doctoral research assistant to study the distribution, spread and general biology of bracken A major thrust of the work will involve the use of field experiements to build predictive models Applicants should be numerate and have some experience in at least one of the following fields bracken ecology, population dynamics of clonal plants, carbon balance studies or ecological modelling

The successful candidate will be based at the Institute of Terrestrial Ecology, Monks Wood and will be expected to have obtained, or be due to obtain, a PhD in an appropriate discipline

The appointment is for three years and salary will be in the range of **£10,678 – £14,909** per annum depending on experience and qualifications A full driving licence is essential The post offers generous holidays and a non contributory pension scheme

The Institute of Terrestrial Ecology is a component body of the Natural Environment Research Council

Further information and application forms may be obtained from Mrs C Rigden, Institute of Terrestrial Ecology, Monks Wood Experimental Station, Abbots Ripton, Huntingdon, Cambs PE17 2LS, or by telephoning (04873) 381 Closing date for applications, and full CV's is 30th November 1989

The Natural Environment Research Council is an Equal Opportunities Employer

Natural
Environment
Research
Council

(2116)A

SANDWELL HEALTH AUTHORITY
THE MIDLAND CENTRE FOR
NEUROSURGERY AND NEUROLO
Holly Lane, Smethwick,
Warley, West Midlands, B67 7

SENIOR BIOCHEMIS

Salary will be within the range £8,2
£11,073 (Basic Grade) or £11,778-£15,
(Senior Grade) Whitley Council Con
tions of Service apply

Applications are invited from gradua
in Biochemistry and related sciences
this full-time vacancy in the Neuroche
istry Section of the Department of Neu
pathology of the above Hospital In
appointment may be at the Basic Gra
for candidates without previous relev
experience The Neurochemistry Labo
tory is the main referral centre for labo
tory investigations of nervous syst
disease, for the West Midlands Inve
gations include the use of HPLC, GC,
and specific protein analysis, and pre
ous experience of these techniqu
would be an advantage The success
candidate will become familiar with
range of investigations undertaken,
cover for other staff, but will chiefly ass
the Principal in extending and develo
ing the repertoire Original work, leadi
to publication, will be actively encourag

The laboratory may be visited by ap
ointment Telephone Mr Westhead (Pr
cipal Biochemist) on 021 558 3232
257 Application form and job descripti
available from the Administrator, at t
above address

Closing date 30th November 1989

Sandwell is a non-smoking health auth
rity (2085)A

The Faculty of Chemistry and Pharmacy,
Institute of Biochemistry, University of Munich
Applications are invited for the tenured position of an

Associate Professor (C3) of Biochemistry

The applicant has to present the field both in teaching (lectures, courses) and research He/she should preferentially be working in the area of biochemistry of development or the structure and function of proteins There is an age limit of 52 years (at the date of appointment) The University of Munich complies with an equal opportunities policy

Applications with the necessary academic qualifications (Ph D , teaching experiences, research record) are to be submitted with a *curriculum vitae, academic records* and *list of publications* to the **Dekan der Fakultat fur Chemie und Pharmazie, Universitat Munchen, Geschwister-Scholl-Platz 1, 8000 Munchen 22, F R G** by January 31, 1990 (W6727)A

UNIVERSITY OF GENEVA FACULTY OF SCIENCE

A professorship (full-professor, possibly assistant-professor) is available in the Department of Zoology and Animal Biology at the University of Geneva

The expected research field is neurobiology or a complementary discipline The new professor will be expected to participate in both undergraduate and graduate teaching and be able to develop an independant and substantial research program

The teaching will be in French and the applicants should have a working knowledge of this language and be prepared to undertake and effort to become fluent within a year

Applications should be sent by February 28, 1990 to **Secrétariat de la Faculté des Sciences, 20, quai Ernest-Ansermet, 1211 Genève 4, Switzerland,** where inquiries can also be addressed (W6722)A

RESEARCH FELLOW

The Natural History Museum is currently expanding its research base into the use of DNA in taxonomic and evolutionary studies Applications are now invited for a three-year post-doctoral appointment in the Experimental Taxonomy Division

The appointee will play a major role in a research programme to be funded by the Wellcome Trust concerned with ribosomal RNA gene sequences of schistosomes Previous experience in molecular biology would be a distinct advantage together with an interest in systematics and evolutionary biology

Starting salary will be linked to the University Grade IA Scale and in the range of £12108- £18315, inclusive of inner London weighting, according to qualifications and experience The appointment will attract pension benefits under the terms of the Principal Civil Service Pension Scheme

Please send a full curriculum vitae with the names and addresses of 2 referees to the Personnel Section, The Natural History Museum, Cromwell Road London SW7 5BD
Informal enquiries to
Dr D Rollinson (01-938 8887)
Closing date 7 December 1989

The Natural History Museum is an equal opportunity employer

THE
NATURAL
HISTORY
MUSEUM

(2104)A

SENIOR SCIENTIFIC OFFICER

(Research)

Human Reproduction Unit
Royal North Shore Hospital
Sydney, Australia

The Human Reproduction Unit has a large group of scientists studying various basic and clinical aspects of the control of the establishment of pregnancy and the development of the early embryo It has well equipped laboratories suitable for the range of techniques in this field The current project requires a research scientist with experience in Cell Biology and/or Pharmacology to study the expression, identify and function of receptors for growth factors, particularly PAF, on the mammalian embryo (Lancet 1989, ii, 769-772) The successful applicant will have a Ph D or equivalent with a demonstrably strong background in a relevant area of research

The position is for 3 years in the first instance with possibility for renewal The salary range is $36,248 – $44,409 p a depending on experience

Enquiries to Dr C O'Neill, Phone 61-2-438 7148 or FAX 61-2-438 7779 and applications in writing to **Personnel Manager**

Closing date 1st December, 1989 (W6721)A

The Department of Neurology at the University of Michigan is seeking applications for a **Research or Tenure Track Position** in Molecular Neurobiology and/or protein chemistry Successful candidate will join a multi-disciplinary team of investigators studying neurotransmitter receptors Position includes laboratory space, start-up funds, and salary support for initial years of program Opportunity exists for a joint appointment in a basic science department Curriculum vitae and three letters of recommendation should be sent to **Robert L Macdonald, MD, PhD, Department of Neurology, University of Michigan, 1108 Neuroscience Bldg , Ann Arbor, MI** 48109-1687 *The University of Michigan is an equal opportunity/affirmative action employer and encourages women and minorities to apply* (NW4503)A

PLANT BREEDING INTERNATIONAL

POST DOCTORAL SCIENTIST and GRADUATE RESEARCH ASSISTANT/ ASSISTANT MANAGER

required in our Biotechnology Group to work on

TOMATO RFLP ANALYSIS

These positions are to support the tomato breeding activities of RAGU Foods Inc (a sister Unilever company) and will become part of the existing PBI Cambridge RFLP group We are looking for

1 an enthusiastic post-doctoral scientist with experience in plant molecular biology/RFLP work who is keen to apply these techniques to tomato breeding and prepared to jointly manage the tomato RFLP programme Althouth based in Cambridge this will involve direct liaison with RAGU breeders/scientists in California (at least one visit per year), and possibly work in US laboratories (Ref RAGU/80)
2 a similarly motivated graduate research assistant to work in the above programme Laboratory experience in molecular biology would be an advantage (Ref RAGU/81)

For further details and application form telephone Cambridge 840411 Ext 376, or write to the **Personnel Officer, Plant Breeding International Cambridge Ltd, Maris Lane, Trumpington, Cambridge CB2 2LQ** quoting the appropriate reference *An Equal Opportunities Employer* (2101)A

PBI
CAMBRIDGE
PLANT BREEDING INTERNATIONAL

RESEARCH FELLOW

(Soil Scientist)

A$31,525 to A$38,567 per annum

Division of Plant Industry, Canberra ACT

THE DIVISION The CSIRO Division of Plant Industry conducts research on problems fundamental to plant biology and agricultural production Prominent among the research activities are programs concerned with gene transfer and expression, cell culture, herbicide biology and synthesis, photosynthetic processes and biological nitrogen fixation

THE PROGRAM The Australian Wool Corporation is funding a project with the aim of maximising the profitability of grazing enterprises while conserving the natural resources This includes work on the nutrition of pastures such as correcting nutrient deficiencies and imbalances and optimising the use of fertilizers in ley farming systems

THE JOB The appointee will undertake research into the possibility of using surface applications of gypsum, phosphogypsum and lime combinations to ameliorate subsurface acidity in soils and define the conditions for successful application

THE PERSON Applicants should have a Ph D or equivalent qualifications with research achievement in science or agricultural science A knowledge of ligard exchange and aluminium speciation in soils is also required

CONDITIONS Appointment will be for a period of 3 years with Australian Government Superannuation benefits available

MORE INFORMATION Prospective applicants are invited to telephone Dr John Freney on 6162 46 5442 for further information A copy of the detailed duty statement and selection criteria may be obtained by telephoning 6162 46 5206

APPLICATIONS Applications should be submitted by 15 December 1989 and quote reference number N8924 They should be framed against the selection criteria, and state relevant personal particulars including details of qualifications and experience Applicants should give details of at least two referees, and address their applications to

The Chief
CSIRO Division of Plant Industry
GPO Box 1600
CANBERRA ACT 2601
AUSTRALIA

(W6725)A

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

CSIRO
AUSTRALIA

AB1 PLN8924

Medical Research Council
Human Genetics Unit

CYSTIC FIBROSIS

A research assistant/post doctoral position is available immediately to join an established cystic fibrosis research team We will use our expertise in gene targetting by homologous recombination (Dorin, Inglis & Porteus (1989) Science, 243, 1357–1360) to create specific CF mutations in the mouse germ line via embryonal stem cells as a route to disease testing and therapy The post which is supported by the Cystic Fibrosis Research Trust is available for two years in the first instance, salary range £10,458-£15,372 Suitable candidates are likely to be experienced in cell culture, with a good grounding in molecular biology Applications quoting ref no CF18 and including a CV along with the names of two academic referees should reach **Ms M Marron, MRC Human Genetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, (031 332 2471)**, by 1st December 1989 The MRC is an Equal Opportunities employer (2100)A

LIFE SCIENTISTS & CHEMISTS

Top quality scientists and laboratory technicians in the following disciplines are required for short-term and permanent positions in a variety of laboratories throughout the United Kingdom

cpl
SCIENTIFIC

- ⋆ CHEMISTRY
- ⋆ FOOD SCIENCE
- ⋆ PHARMACOLOGY
- ⋆ TOXICOLOGY
- ⋆ BIOCHEMISTRY
- ⋆ MICROBIOLOGY

Register with the specialist employment agency at no cost

Employers, if you need permanent staff, temporary cover or additional help without commitment why not contact us?

CPL Scientific Employment Services
Science House, Winchcombe Road, Newbury, RG14 5QX, England
Telephone 0635 524064 (24 hour answering)

(9881)A

CHAIR

ENVIRONMENTAL TOXICOLOGY GRADUATE PROGRAM

UNIVERSITY OF CALIFORNIA, RIVERSIDE

Nominations and applications are invited for the Chair of the Environmental Toxicology Graduate Program at the University of California, Riverside Candidates are expected to have an internationally recognized research program utilizing biochemical, cellular and/or molecular approaches towards understanding fundamental mechanisms of toxic action in biological systems In addition, the Chair must have demonstrated teaching excellence, administrative ability, leadership and enthusiasm to administer an interdepartmental program while continuing an active research program

The Interdepartmental Environmental Toxicology Graduate Program is administered from the Department of Entomology and includes participating faculty from the Departments of Biochemistry, Botany and Plant Sciences, Chemistry, Nematology, Plant Pathology, Soil and Environmental Sciences, the Division of Biomedical Sciences and the Statewide Air Pollution Research Center The current research strength of faculty in this Program is on the biochemical, cellular and molecular mechanisms of toxicity of agricultural chemicals and other environmental toxicants, and on the fate and transformation of these toxicants in the environment

The appointment of the Chair, whose major research and teaching responsibility is to the Environmental Toxicology Graduate Program, will be at the tenured Full Professor level in the Department of Entomology Teaching responsibilities will include graduate-level instruction and supervision of graduate students in the Environmental Toxicology Program The appointees will also have the opportunity to be involved in other Interdepartmental Graduate Programs on campus

Submit a detailed curriculum vitae, list of publications, representative reprints, a statement of research interests, and names and addresses of six referees to

S S Gill, Search Committee Chairman, Department of Entomology and the Environmental Toxicology Graduate Program, University of California, Riverside, CA 92521 Applications and nominations accepted until February 1, 1990

The University of California is an Equal Opportunity/Affirmative Action Employer, and Minorities and Women are particularly encouraged to apply (NW4590)A

CLINICAL PHARMACOLOGIST - PHYSIOLOGICAL PHARMACOKINETICIST

The Laboratory of Clinical Studies, National Institute on Alcohol Abuse and Alcoholism, Public Health Service, invites applications for one Clinical Pharmacologist - Physiological Pharmacokineticist This position is offered depending on the experience of the applicants at the level of a senior staff fellow (salary range, $28,000 - $45,234) The Laboratory is involved in exciting studies on brain imaging and drug metabolism The individual appointed will have an important role in this multidisciplinary team effort A research ward, excellent analytical chemistry, brain imaging and computer resources are available in the Laboratory on the NIH campus Position information and qualification requirements may be obtained by contacting Dr Linnoila Submit Curriculum Vitae and names of three references to

Markku Linnoila, M D , Ph D
Clinical Director
National Institutes of Health
Building 10, Room 3B-19
9000 Rockville Pike
Bethesda, MD 20892
(301) 496-9705

This position is now available

NIH IS AN EQUAL OPPORTUNITY EMPLOYER (NW4533)A

POSTDOCTORAL POSITION

HARVARD UNIVERSITY

A postdoctoral position in theoretical atmospheric chemistry is available immediately The successful candidate will join a large research group conducting theoretical and experimental investigations of a broad range of issues in atmospheric chemistry He/she will participate in current efforts to simulate global tropospheric chemistry using a general circulation model He/she will also have opportunities to become involved in other projects, including photochemical model studies and analyses of field data Independent research will be encouraged

A strong background in atmospheric science, chemical engineering, environmental engineering, or physical chemistry is required Previous experience in scientific computing is a plus

Interested persons should send a curriculum vitae and the names and addresses of three references by December 31, or until position is filled, to **Dr Daniel Jacob, Department of Earth and Planetary Sciences, 29 Oxford St, Harvard University, Cambridge, MA 02138**

Harvard University is an equal opportunity/affirmative action employer (NW4575)A

ASSOCIATE TOXICOLOGIST

The California State Department of Health Services is recruiting for Associate Toxicologist for immediate openings An employment examination is scheduled for fall, 1989 The examination will be based 100 per cent on interviews Telephone interviews are permissible Vacancies exist in Sacramento, Emeryville, Berkeley, and Los Angeles The areas of involvement will be environmental toxicology and risk assessment The annual salary will start at $38,304-$50,748 To qualify for the Associate Toxicologist exam, you must have a Doctoral Degree in toxicology, or a closely related specialty, or possession of a Masters Degree in toxicology or closely related speciality and three years experience, past the Masters Degree, in the area of toxicology, or certification as a Diplomate of the American Board of Toxicology Please send your resume to

State Department of Health Services
Office of Environmental
Health Hazard Assessment
714 P Street, Room 442
Sacramento, CA 95814
ATTN Donna Rowe (NW4588)A

DEPARTMENT OF HEALTH SERVICES

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE

(University of London)

MOLECULAR CELL PATHOLOGY UNIT

1) Postdoctoral Research Fellow, Research Scale II from £15,372 + £1650 L A

2) Research Assistant IB from £9,816 + £1650 L A

The unit has two Wellcome Trust fund vacancies to join a multidisciplinary team investigating molecular mechanisms of differentiation Post 1 is for 5 years and Post 2 is for 4½ years The new team will be applying a novel strategy to clone differentiation genes, initially in haemopoietic cells The gene probes generated will be used to investigate the role of DNA breakage/reunion reactions of DNA topoisomerases in differentiation

Applicants should have experience in molecular biology

For informal enquiries please contact Drs G E Francis and D Fisher (01-794 0500, Exts 4904 or 3430)

Further details can be obtained from the **School Office, R F H S M , Rowland Hill Street, London NW3 2PF** (01-794 0500, Ext 4262), to which applications should be sent by 1st December 1989 Candidates should submit a curriculum vitae (3 copies) with names and addresses of two referees Please state clearly in any enquiry/application the number of the post in which you are interested *Please quote REF MCU/PATH/58* (2028)A

Medical Research Council

Human Genetics Unit

RESEARCH POSITIONS IN HUMAN MOLECULAR GENETICS

Vacancies exist for 2 post-doctoral scientists to work in teams concerned with the isolation and application of DNA markers in a study of genes involved in inherited mental illness, eye disorders and cancer predispositions, and on detecting mutation and recombination in single cells The appointments are for a limited term of 3-5 years Salary scale £10458-£15372 Further details from **Ms M Marron, MRC Human Genetics Unit, Western General Hospital, Crewe Road, Edinburgh EH4 2XU, (031 332 2471), and applications, with the names of two referees, by December 18, 1989** The MRC is an Equal Opportunities employer (2099)A

Genetics of Mental Illness

Assistant/Associate Research Scientist in the Mental Health Research Institute and Assistant/Associate Professor of Human Genetics

Applications are invited for a joint tenure-track appointment in the Mental Health Research Institute in collaboration with the Departments of Psychiatry and of Human Genetics at the University of Michigan Medical Center Applicants should have a Ph D or M D and research interest in the genetics of mental illness/behavioral disorders The candidate selected will be expected to establish a vigorous, independent, externally funded research program in the genetics of human nervous system function and mental illness As an active member of the Departments of Human Genetics and Psychiatry and the Institute, the appointee may participate in several outstanding graduate and medical programs Applicants should submit their curriculum vitae, a description of their research plans and the names of three references to **Dr S J Watson, Mental Health Research Institute, 205 Washtenaw Place, University of Michigan, Ann Arbor, MI 48109.** The Universty of Michigan is an Equal Opportunity Employer Please reply by January 30, 1990

The University of Michigan

A non-discriminatory affirmative action employer

(NW4593)A

CELL BIOLOGY

SENIOR LEVEL FACULTY POSITION

DEPARTMENT OF BIOLOGY
UNIVERSITY OF CALIFORNIA, RIVERSIDE

Applications are invited for a tenured senior level (Associate/Full Professor) faculty position in the Department of Biology Preference will be given to scientists applying modern molecular approaches to the study of the cell All areas of cell biology will be considered However, we are particularly interested in individuals concerned with cell–cell interactions, protein sorting and assembly, cytoskeletal structure and function, or the extracellular matrix

Applicants should have an active research program and an established reputation with a proven record of competing successfully for extramural funding Demonstrated interest and ability in teaching is sought Salary will be competitive and will depend on qualifications and experience

Modern research space and access to major equipment such as TEM, SEM, and staffed biotechnology and analytical chemistry instrumentation facilities are available

Applications must be received by January 15, 1990 Please send *curriculum vitae*, summary of research interests and arrange to have letters sent by three to five referees to

Chair,
Cell and Molecular Biology Search Committee,
Department of Biology,
University of California, Riverside, CA 92521

The University of California is an Equal Opportunity/ Affirmative Action Employer and minority and female candidates are encouraged to apply (NW4589)A

Physical Oceanographer

Department of Physical Oceanography seeks applicants for appointment to Assistant, Associate or Senior Scientist level depending upon background, experience and tenure suitability Ph D in physical oceanography or related field, postdoctoral experience and demonstrated expertise in oceanography preferred Experience in acquisition and analysis of observations or in development of dynamical models of ocean processes desired

Send curriculum vitae, short statement of research interest and publication list by April 15, 1990 to

Dr. Robert R. Beardsley
Chairman, Department of
Physical Oceanography
Box 54P

WOODS HOLE OCEANOGRAPHIC INSTITUTION

Woods Hole, MA 02543

An equal opportunity employer M/F/H/V

(NW4571)A

FACULTY POSITION IN NUMERICAL MODELLING

The University of Miami invites applications for an academic track faculty position in the Rosentiel School of Marine and Atmospheric Science, Division of Meteorology and Physical Oceanography Rank and salary will be negotiated according to qualifications minimum requirements are a Ph D degree in physical oceanography or closely related discipline, and demonstrated independent research capability Graduate level teaching competence in fluid mechanics with emphasis on numerical models of meso- and/or basin-scale dynamics is desirable

The appointee in this position is expected to develop individual research activities which complement an active program of ocean dynamics research based on in situ field observations, satellite remote sensing, and research at NOAA's Atlantic Oceanographic and Meteorological Laboratories, which are located nearby A central interest in applications of model results to the analysis and understanding of ocean circulation and coupled systems is particularly appropriate, as would be an interest in personal participation in field studies

Send curriculum vitae, publication list and names of three references by 15 January 1989 to **Chairman, Physical Oceanography Search, Division of Meterology and Physical Oceanography, Rosentiel School of Marine and Atmospheric Science, University of Miami, 4600 Rickenbacker Causeway, Miami, Florida 33149–1098**

The University of Miami is a private, independent, international University and an Affirmative Action/Equal Opportunity Employer (NW4591)A

UNIVERSITY OF ZIMBABWE

Applications are invited for the following posts

DEPARTMENT OF BIOLOGICAL SCIENCES

The successful applicant will teach undergraduate courses in Cell Biology and contribute to Honours and postgraduate programmes

DEPARTMENT OF GEOLOGY (2 posts)

Post A: SEDIMENTOLOGIST

The successful applicant will teach courses in sedimentology, applied palaeontology and stratigraphic analysis

Post B: SEDIMENTARY PETROLOGIST

Preference will be given to applicants with a PhD and an ability to teach sedimentology at all levels as well as supervising higher degree candidates

Salary scale Lecturer Z$22,488-Z$31,692, Senior Lecturer Z$32,736-Z$35,436, Associate Professor Z$36,492-Z$37,680

Appointment on the above scales will be according to qualification and experience

CONDITIONS OF SERVICE Both permanent and short-term contracts are offered Persons who are not Zimbabwean citizens may be appointed only on a short-term contract basis for an initial period of two years Short-term contract may be extended

Six copies of applications quoting ref ASA/17/10/89 and giving full personal particulars which should include full name, place and date of birth, qualifications, employment and experience, present salary, date of availability, telephone number and names and addresses of three referees should be addressed to **Deputy Registrar (Administration), University of Zimbabwe, P O Box MP 167, Mount Pleasant, Harare, Zimbabwe,** (Telegrams UNIVERSITY Telex 4-152 ZW)

Closing date for receipt of applications for the posts is *7 December 1989*

Applicants from outside Zimbabwe should also send a copy to the **Appointments Officer, Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF, UK,** from whom further information may be obtained (W6719)A

ASSISTANT PROFESSOR OF INORGANIC CHEMISTRY

The Department of Chemistry University of Winnipeg, invites applications for a tenure-track appointment to begin July 1990 A Ph D in Inorganic Chemistry is required, preferably with post-doctoral research experience The University is primarily an undergraduate institution committed to excellence in teaching and research The responsibilities include teaching inorganic chemistry, the accompanying laboratory, and some introductory chemistry, as well as the development of a research program

Applications consisting of a curriculum vitae, outline of research interests, and names and addresses of three referees should be sent, by January 15, 1990, to **Dr H M Hutton, Chairman, Department of Chemistry, University of Winnipeg, Winnipeg, Manitoba, Canada, R3B 2E9** *In accordance with Canadian Immigration regulations, this advertisement is directed to Canadian citizens and permanent residents of Canada* (NW4580)A

THE WELLESLEY HOSPITAL RESEARCH INSTITUTE AND DEPARTMENT OF IMMUNOLOGY UNIVERSITY OF TORONTO

Applications are invited for the position of

LEADER

of a new basic research initiative to investigate cellular and molecular aspects of immunoregulation and autoimmunity

Applicants (Ph D or M D) for the program leader should be at the Associate or Full Professor level

The leader will be responsible for recruiting three additional individuals at the Assistant or Associate Professor (Ph D or M D) level Salary commensurate with experience The Institute will provide ample space and generous start-up funds in addition to substantial ongoing administrative funding Consideration in the first instance will be given to Canadian citizens Application deadline is January 1, 1990

Enquiries and curriculum vitae should be directed to **Dr E C Keystone, c/o Research Office, The Wellesley Hospital, University of Toronto, 160 Wellesley St East, Toronto, Canada M4Y 1J3** (NW4596)A

Pediatrician/immunogeneticist/molecular biologist

A position is available as Research Assistant Professor in the Department of Microbiology, Southwestern Medical Center The ideal candidate would have a background in pediatrics and have at least three years postdoctoral experience in immunogenetics/molecular biology Modest patient contact, 90% laboratory protected time to study the development of the human immune repertoire Experience with EBV transformation, polymerase chain reaction, cloning & sequencing of DNA, idiotype analysis, etc Send curriculum vitae and two letters of recommendation to **Dr J Donald Capra, Department of Microbiology, The University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd , Dallas, Texas 75235** *The University of Texas Southwestern Medical Center is an equal opportunity employer* (NW4582)A

MEDICAL RESEARCH COUNCIL

RADIOBIOLOGY UNIT, OXON

Cell and Molecular Biology Division

MOLECULAR/CELLULAR BIOLOGISTS

Applications are invited for two non-clinical scientific posts One of these posts is a SHORT TERM appointment of 5 years, while the other may be filled by either a SHORT TERM appointment of 5 years or a CAREER appointment according to the age and experience of the successful candidate A career appointment will be tenable at this Unit in the first instance

The successful candidates will join a group studying molecular and cellular aspects of DNA repair and radiation mutagenesis, chromosome replication and aberration induction and the biophysics of radiation damage Excellent research facilities are available and the Unit also includes groups studying mammalian genetics, radiation leukaemogenesis, cancer therapy and radiation chemistry The postholders will be required to continue and expand the current programme on DNA repair and mutagenesis, in addition, a career appointment will develop his/her own programme of research and promote studies involving molecular biology

Salary on scales equivalent to those for University non-clinical academic staff, Grade 2/1 £12,381-£20,469, or for Senior Grade up to £24,285pa Pension provision The Unit is situated in a pleasant rural area a few miles from Oxford Hostel accommodation available

Please send applications, including full c v , publication list and the names and addresses of 2 referees, to the Personnel Officer, MRC Radiobiology Unit, Chilton, Didcot, Oxon, OX11 0RD Please telephone Dr John Thacker on (0235) 834393 for further information Closing date 15th December 1989

The Council is an Equal Opportunity Employer (2092)A

UNIVERSITY OF DURHAM

DEPARTMENT OF ARCHAEOLOGY

IMAGING ARCHAEOLOGICAL STRUCTURES BY ELECTRICAL RESISTIVIT TOMOGRAPHY

(Re-advertisement)

Applications are invited for a Po doctoral Research Assistant Imaging Archaeological Structu by Electrical Resistivity Tom raphy for a research project fund by SERC in conjunction w Geoscan Research

Geophysical surveys now pla crucial role in archaeological c servation, excavation plann and resource assessment Exist techniques generally provide o 2-D area maps over buried featu with minimal depth informati This project will transfer rec developments in medical imag technology to archaeology by veloping a novel electrical resist ity tomography system capable resolving archaeological structu in 3-D The research will invo theoretical studies, numer simulation, laboratory and fi experiments with interaction tween the archaeological, med and physical sciences Suitable c didates will have a strong ba ground in applied mathemat physics, geophysics or related fiel

The appointment is tenable three years from as soon as poss and the initial salary will be on Research Staff Scale Grade (£10,458-£12,381 p a) Inform enquiries may be made to Dr Noel, Department of Archaeolo (091) 374 3631

Further particulars (quot reference SS/AS/89 04) may obtained from the Registrar a Secretary (Faculty of So Sciences), University of Durha Old Shire Hall, Old Elvet, Durh DH1 3HP, telephone (091) 2995, to whom applications (th copies), including the names a addresses of three referees, sho be sent not later than Wednesda December 1989 Previous can dates need NOT apply (2074)A

FACULTY POSITIONS IN PHYSICAL OCEANOGRAPHY

The University of Miami invites applications for academic and research faculty positions (one each) in the Rosenstiel School of Marine and Atmospheric Science, Division of Meteorology and Physical Oceanography Rank and salary will be negotiated according to qualifications Minimum requirements are a Ph D Degree in physical oceanography or a closely related discipline, and demonstrated independent research capability Graduate level teaching competence in fluid mechanics with emphasis on large scale dvnamics or on boundary layer processes is desirable for the academic position

The appointees in these positions are expected to develop individual research activities which complement an active program of ocean dynamics research based on in situ field observations, satellite remote sensing, and researchers in NOAA's Atlantic Oceanographic and Meteorological Laboratories, which are located nearby A central interest in applications of space-based observing techniques to oceanic studies and dynamic analysis based on either, or a combination of, field experiments and model simulations, is particularly appropriate, as would be an interest in personal participation in field or numerical model studies

Send curriculum vitae, publication list and names of three references by 15 December 1989 for the research position and 15 January 1990 for the academic position to **Chairman, Physical Oceanography Search, Division of Meteorology and Physical Oceanography, Rosenstiel School of Marine and Atmospheric Science, University of Miami, 4600 Rickenbacker Causeway, Miami, FL 33149-1098**

The University of Miami is an Affirmative Action/Equal Opportunity Employer (NW4592)A

UNIVERSITY OF MICHIGAN

Invertebrate Paleontologist

The Museum of Paleontology and Department of Geological Sciences invite applications for a joint tenure-track position in invertebrate paleontology The position will be at the rank of ASSISTANT PROFESSOR, with appointment to be made in 1990 PhD is required We seek applicants with broad research and teaching interests in paleobiology, and expertise in the systematics of fossil invertebrates (metazoan and/or protistan) Current faculty in paleontology are C B Beck, D C Fisher, P D Gingerich, and G R Smith Resources include strong programs in organismal and evolutionary biology within the university Museum appointment involves shared curatorial responsibility for a major invertebrate research collection Interested candidates should send a resume, names of five persons from whom the Museum and Department may request letters of recommendation, and a brief statement of research interests, to **Prof Daniel C Fisher, Search Committee Chairman, Museum of Paleontology, The University of Michigan, Ann Arbor MI 48109-1079, U S A** Closing date for applications is January 31, 1990 The University of Michigan is a non-discriminatory, affirmative action employer (NW4594)A

STAFF TOXICOLOGIST

The California State Department of Health Services is recruiting for Staff Toxicologist for immediate openings An employment examination is scheduled for fall, 1989 The examination will be based 100 per cent on interviews Telephone interviews are permissible Vacancies exist in Sacramento, Emeryville, Berkeley, and Los Angeles The areas of involvement will be environmental toxicology and risk assessment The annual salary will start at $50,748-$61,392 To qualify for the Staff Toxicologist exam, you must have a Doctoral Degree in toxicology, biochemistry, pharmacology or a closely related specialty and three years of postdoctoral experience in toxicology Please send your resume to

State Department of Health Services
Office of Environmental
Health Hazard Assessment
714 P Street, Room 442
Sacramento, CA 95814
ATTN Donna Rowe (NW4587)A

University of Manchester

DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

POST-DOCTORAL RESEARCH ASSOCIATE

Applications are invited for a two year post available from January 1990 in an active research group examining the molecular basis of cellular interactions with the extracellular matrix The position is funded by The Wellcome Trust for identification and characterisation of chondrocyte intergrin adhesion receptors and will involve approaches spanning the areas of molecular biology, cell biology and synthetic peptide technology Salary range £10,458 £16,665 p a (Research IA)

Written applications, including a full c v and names and addresses of two referees, to **Dr Martin J Humphries, Department of Biochemistry and Molecular Biology, Stopford Building, The University, Manchester M13 9PT** Closing date December 1st, 1989

The University is an equal opportunities employer (2088)A

STANFORD UNIVERSITY

seeks a distinguished

SENIOR OLD WORLD ARCHEOLOGIST

with expertise in the last 300,000 years of human evolution to provide leadership for a new Program in Human Biocultural Evolution within the Anthropology Department and to teach in the Human Biology Program For more information contact **Dr Harumi Befu, Department of Anthropology, Stanford University, Stanford, California 94305-2145** Application deadline is January 1, 1990 *Stanford is an Affirmative Action/Equal Opportunity Employer* (NW4583)A

Postdoctoral position in Molecular Immunology

One position is available for the study of regulation of immunoglobulin synthesis in human primary immunodeficiency The department is a major referral center for patients with various forms of immunodeficiency Research is mainly focussed on aspects of immunoglobulin deficiency Work includes mapping and sequencing of immunoglobulin genes and gene deletion breakpoints Send C V and names of references to **CIE Smith & L Hammarstrom, Center for BioTechnology, Karolinska Institute, NOVUM, S-141 52 Huddinge, Sweden** Tel Sweden-8-7795204 FAX-8-7466869 (W6713)A

THE AFRC INSTITUTE OI HORTICULTURAL RESEARCH

East Malling

HIGHER SCIENTIFIC OFFICER (Temporary)

Crop & Environment Protectic Department

Transgenic Plants — Risk Assessment
(up to 3 yr appointment)

A temporary Higher Scienti Officer exists, funded for up to years by the Department of t Environment, for a populatic biologist with an interest in pla genetics to study the risks ass ciated with the agricultural explc tation of transgenic plants usi rapid-cycling Brassica species as model system The person appoi ted will work with Dr Ian Crute (IHR, East Malling) and in col boration with Dr John Barra (University of Cambridg Department of Genetics) T work will involve the selection ar breeding of plant lines carryi appropriate dominant mark genes (particularly disease res tance genes) and the establis ment of rates of gene flow with and between Brassica speci under controlled conditions and the field

Qualifications First or Upp Second Class honours degree in biological science including a su stantial component of genetic plant breeding and population bi logy, plus 2 years relevant pos graduate experience, knowled of plant pathology an advantage

Salary In range £10,67 £14,335

Further details and applicatic form from the Personnel Office Institute of Horticultural R search, Bradbourne House, Ea Malling, Maidstone, Kent ME 6BJ Ref 95/EM Closing date 7 December 1989

The AFRC is an Equal Oppor unities Employer (2106)A

UNIVERSITY OF SOUTHAMPTON

Institute of Cryogenics

Postdoctoral Research Assistant required to develop engineering applications of high temperature superconductors Previous experience desirable Salary up to £11,088 Applications should be sent to **Mr H F Watson, Staffing Department, The University, Southampton, SO9 5NH, telephone (0703) 595000 ext 2706,** including the names of 2 referees Please quote reference number 139/HFW/SMT Closing date 4th December 1989 (2105)A

DIRECTOR

Institute for Astrophysics
Dept of Physics & Astronomy

University of New Mexico

Please see Nature Sept 21 1989 p 17 *EOE/AA* (NW4585)A

UNIVERSITY OF GLASGOW

Post-doctoral Scientist : Haemopoiesis in sheep

A post-doctoral position is available to study the aetiology of haemopoietic disorders in helminth infections in ruminants The successful candidate will join a multidisciplinary research group elucidating the basis of genetic resistance of sheep to helminth parasites Experience of research into control of growth and regulation of mammalian cells, especially haemopoietic cells is important Previous experience of research on the aetiology of anaemias would be advantageous

The appointment will be for three years in the first instance Salary scale £10,458–£12,381 per annum

Further information may be obtained from **Professor Max Murray, Department of Veterinary Medicine/or Professor G M Urquhart, Department of Veterinary Parasitology, University of Glasgow Veterinary School, Bearsden Road, Bearsden, Glasgow G61 1QH Telephone 041–339–8855** (2084)A

UNIVERSITY OF ROCHESTER

POSTDOCTORAL POSITION

The succesful applicant will work on a project forming part of a multidisciplinary program focusing on the regulation and structure-function relationship on anion transport pathways in exocrine glands Experience in the physiology of ion transport pathways and a willingness to learn to apply molecular biological techniques to the study of these pathways is preferred Please submit a curriculum vitae and two references to

Dr James E Melvin
School of Medicine and Dentistry
University of Rochester
601 Elmwood Avenue, Box 611
Rochester, NY 14642 (NW4579)A

UNIVERSITY OF MANCHESTER

Department of Medicine

POST-DOCTORAL
ESEARCH ASSOCIATE

his post is offered on a hree-year project supported y the Wellcome Trust This s a collaboration with the aboratoire Central de Genie enetique, Liege, Belgium, o study promoter elements f the human prolactin gene nd their interaction with uclear trans-acting factors nvolved in intracellular regu-ation of transcription The roject will involve transfec-ion assays, gel retardation nalysis and DNase footprint-ng, and applicants should ave previous practical ex-erience of molecular bio-ogical techniques Initial alary range per annum 10,458-£12,879 Applica-ions to **Dr J R E Davis, epartment of Medicine, topford Medical Building, niversity of Manchester,** tel 61-275 5593/5182 from hom further details are vailable *The University is n equal opportunities mployer* (2115)A

UNIVERSITY OF SASKATCHEWAN

Department of Biology

Postdoctoral Position

Applications are invited for a postdoctoral position in insect reproductive physiology Candidates should have expertise in modern *biochemical* techniques, and some knowledge of insect tissue culture would be advantageous The successful applicant will participate in an ongoing study of the regulation and functions of reproductive activity in the male insect Salary will be in accord with NSERC Canada guidelines (current range Can$21,500-23,000 per annum) Please send *curriculum vitae* and names/addresses of three referees to

Prof C Gillott
Department of Biology
University of Saskatchewan
Saskatoon, SK S7N 0W0
Canada

For further information, phone (306) 966-4401 (NW4595)A

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nature

Still first with the best in science

UNIVERSITY OF ABERDEEN

DEPARTMENT OF GENETICS AND MICROBIOLOGY

MOLECULAR BIOLOGIST

Applications are invited for a three-year Postdoctoral Fellowship for an AFRC funded position on the Identification and Isolation of Hypervariable DNA loci in Arabidopsis thaliana Applicants should have experience in Recombinant DNA techniques or in genomic cloning Experience in eukaryotic molecular biology would be an advantage The project is part of a major AFRC initiative on Arabidopsis molecular genetics and involves collaboration with other members of the program Salary within the range £10,458-£16,665 on the Research and Analogous Scale IA (postdoctoral)

Further particulars from the **Personnel Office (tel 0224 273500)** Applications by letter with CV including the names of three referees to the **Personnel Office, The University, Regent Walk, Aberdeen AB9 1FX** by 8 December 1989 quoting reference number JA/073

(2103)A

UCLA

POST-DOCTORAL POSITIONS

Immediate opening for 2 postdoctoral fellows in active research group studying aging One position involves molecular biological investigation of gene expression during aging Second position involves studies of senescence changes in long-term T lymphocyte cultures and requires background in molecular biology, immunology &/or biochemistry, and experience in tissue culture techniques Send Curriculum Vitae, statement of research interests and reference names to **Dr Roy Walford, Department of Pathology, UCLA Medical School, Los Angeles, CA 90024** *UCLA is an equal opportunity/affirmative action employer* (NW4584)A

FELLOWSHIPS

Postdoctoral Fellowship Position

Molecular Immunology and Virology

A postdoctoral position is immediately available for the study of either the molecular biology of HTLV-1 and HIV-1, structure-function relationships in the human interleukin-2 receptor or DNA binding proteins that regulate transcription of T cell activation genes Prior experience in molecular biology and/or biochemistry is required

Interested applicants should send curriculum vitae and names of three references to **Dr Dean W Ballard, c/o Dr Warner C Greene, Howard Hughes Medical Institute, Duke University Medical Center, P O Box 3037, Durham, North Carolina 27710** or Telefax 919-684-5458

(NW4567)E

continued on page 16

STUDENTSHIPS

DURHAM UNIVERSITY/SHELL RESEARCH LTD

RESEARCH STUDENTSHIPS/ VIBRATIONAL SPECTROSCOPY

Investigations of interactions of polymer surfactants at solid/liquid Interfaces

This project is related to understanding how polymer surfactants cause stabilised dispersions of soot (and other particles) in automative fuels and lubricants The degree and nature of adsorption of relevant model detergents on a variety of substrates (carbon, silica etc) will be studied using infrared reflection and photoacoustic spectroscopy These measurements, backed up with oscillation rheology and photon correlation spectroscopy, will be employed to relate chemical (molecular) structure with adsorption efficiency In this way it is hoped to build up a better picture of factors influencing the stabilisation of suspensions The project will also include some work on the adsorption of lubricant (surfactant) additives on metal surfaces — in connection with the control of anti-wear properties

Applicants from EEC countries should have a first degree (or equivalent diploma) in Chemistry, Physics or Chemical Physics The studentship attracts a grant of £3,725 p a , (tax free) plus an additional £550 per annum subject to report There will be an opportunity to work at a modern industrial research laboratory (Shell Research Laboratories, Thornton Research Centre, Nr Chester) Applications to Dr J Yarwood (Tel 091-374-3136 or FAX 091-374-3741 Chemistry Department, Durham University, South Road, Durham DH1 3LE, U K as soon as possible (2094)F

continued on page 16

University of Tromsø

HEART RESEARCH

The University of Tromsø has an immediate opening for
A PROFESSOR OF PHYSIOLOGY
until January 1, 1993, with possibility of extension The position is attached to the Department of Medical Physiology which concentrates its activity on experimental heart research This includes studies of cardiac electrophysiology and pharmacology, heart failure, arrhythmias and antiarrhythmic drugs, hemodynamics, ischemia and reperfusion, and hypothermia The Department is equipped for in vitro studies of isolated heart cells and heart tissues, as well as in vivo studies of anesthetized dogs and other animals Applicants are expected to strengthen this research, and must therefore possess research experience and supervising ability in experimental heart research Lectures may be held in English, but as the position also entails administrative work, the successful applicant is expected to learn Norwegian

The Department of Medical Physiology has 2 professors, 2 assistant professors plus research fellows (approx 10) and technical support staff (approx 6)

The gross salary is at present NOK 276 792 per year Applications including a complete c v and list of publications should be sent in 5 copies together with the relevant certificates to
The University of Tromsø, The University Director, Breivika, N-9000 TROMSØ, NORWAY.
Closing date for applications 29 December 1989
Full directives for application and further information about the professorship can be obtained at the University at the abovementioned address, or from Professor Helge Refsum, Institute of Medical Biology, University of Tromsø, Teoribygget, N-9000 Tromsø, NORWAY Tel 47 83-44693 , (W6732)A FRANTZ HRA 89

STUDENTSHIPS continued

UNIVERSITY OF ST ANDREWS
DEPARTMENT OF BIOLOGY & PRECLINICAL MEDICINE
NERC STUDENTSHIP

Applications are invited for a three year studentship, leading to a Ph D , to investigate antibacterial immunity in marine invertebrates The project will be concerned with the interaction between marine micro organisms and marine decapod crustaceans and will be based at the Gatty Marine Laboratory, St Andrews The studentship, supervised by Dr V J Smith, is available immediately

Applicants should have an interest in marine microbiology or the physiology of marine animals and should possess a First or Upper Second Class Honours degree in Biology, Microbiology or another appropriate subject Further information can be obtained from **Dr V J Smith, Gatty Marine Laboratory, University of St Andrews, Fife, KY16 8LB, (tel (0334) 76161 ext 7270)**, to whom applications should be sent by 5th December 1989 (2097)F

THE ROYAL SOCIETY
JOHN MURRAY TRAVELLING STUDENTSHIP IN OCEANOGRAPHY AND LIMNOLOGY 1990

Applications are invited for the above Studentship, open to British graduates under the age of 35, for the encouragement of travel and work in oceanography or limnology This award, which may be held for periods from three months to one year, will in 1990 be up to £2500, adjusted according to qualifications and place and duration of research Applications are especially invited from workers in oceanography and limnology, as well as in zoology, botany, geology, mathematics and physics, and it will be permitted to hold the award while receiving a salary from a regular appointment

Applications receivable by 15 December 1989 should be made on forms obtainable from **Mrs U M A Tokle, The Royal Society, 6 Carlton House Terrace, London, SW1Y 5AG** (1927)F

FELLOWSHIPS continued

FEBS LONG-TERM FELLOWSHIPS

These Fellowships are intended to support one year stays of members of FEBS constituent Societies in a laboratory of another country within the FEBS area There is a possibility of renewal for a further one year period The amount of money allocated per Fellowship will be up to 32000 DM plus travel expenses to and from the receiving laboratory and town of origin A family help up to 3600 DM may be provided

Eligible candidates must have been members of a FEBS constituent Society for at least one year before the application is submitted They should be normally less than 35 years old at the time of application Applications to work in a laboratory in the same country in which the applicant is working or normally works are not considered

Applications shall be received by the Chairman of the Fellowships Committee before April 1st Fellowships will be awarded during the next FEBS Meeting (Budapest, August 1990) Retrospective applications cannot be considered

Application forms and additional information may be obtained from the Chairman of the Fellowships Committee Dr C Gancedo, Instituto Investigaciones Biomédicas CSIC, C/Arturo Duperier 4 E-28029 Madrid, Spain (W6712)E

GULBENKIAN INSTITUTE OF SCIENCE
Oeiras, Portugal
GULBENKIAN RESEARCH FELLOWSHIPS IN BIOLOGY

The Department of Science of the Calouste Gulbenkian Foundation is offering two research fellowships for postdoctoral scientists wanting to work at the Gulbenkian Institute of Science (Biology), Oeiras, Portugal Candidates should be able to work in one of the following areas

- ★ *Neurobiology of purines*
- ★ *Membrane transport in yeasts*
- ★ *Molecular yeast taxonomy*
- ★ *Molecular Biology of stress in yeasts*
- ★ *Structure and function of P450 genes and nRNA in the liver*
- ★ *Mathematical methods in neurodynamics and neuromuscular control*

The fellowships are for two years The stipend is 200 000 portuguese escudos per month Travel assistance may be provided

Applications should be submitted in duplicate to the **Director, Gulbenkian Institute of Science, Apartado 14, 2781 Oeiras Codex, Portugal** They should include a curriculum vitae, the area of research selected from one of those indicated above, and the names and addresses of two referees The closing date is 31 of December (W6720)E

GONVILLE AND CAIUS COLLEGE, CAMBRIDGE
MEDICAL RESEARCH FELLOWSHIP

Gonville and Caius College intends to elect a Research Fellow in some field of medical research Candidates should be medical graduates who are registered with the General Medical Council and will normally be below the age of 35 Candidates will be expected to have undertaken sufficient previous research to be able to demonstrate their research ability The Fellowship will be tenable for up to four years and is particularly directed towards those intending to pursue a career in medical research

The stipend of the Research Fellow will be related to the University's clinical lecturer scale (currently £14,545 – £25,500) if the Fellow is engaged in research involving clinical responsibility and otherwise to the University's senior clinical research associate scale (currently £15,105 – £19,310) Initial placement will depend on age and experience There will be a deduction if the Fellow is resident in College Where appropriate, the College intends to negotiate a suitable honorary clinical contract with the Health Authority

The Research Fellow will receive the normal privileges of a Fellow of the College, including High Table meals and other allowances The College can assist its Research Fellows by contributing towards research expenses

Further details can be obtained from the **Master's Secretary, Gonville and Caius College, Cambridge CB2 1TA** Applications must be received by 15 January (2091)E

The Royal Society

UNIVERSITY RESEARCH FELLOWSHIPS

The Council of the Royal Society invites applications for about 28 appointments to be supported from the Society's Parliamentary Grant-in-aid and tenable from 1 October 1990 as Royal Society University Research Fellowships

All applicants must have a Ph D or equivalent research experience, they must be at least 26 but should not have passed their 33rd birthdays by 1 October 1990 In an exceptional case, however, an award may be made to an applicant who has passed his/her 33rd birthday by this date but who has outstanding ability for ndependent scientific research and who is able to demonstrate that there are special reasons why he/she should still be eligible for a fixed-term research fellowship Applicants must be British Citizens of the United Kingdom) and must hold the appointments in departments of science (including agriculture, medicine, mathematics, engineering and technology) in universities or polytechnics in the United Kingdom Persons already holding substantive posts in universities or polytechnics in the United Kingdom will not normally be considered

Appointments, which will be subject to the Society's General Regulations governing Royal Society Research Appointments, will be for up to five years with the possibility of renewal for two successive periods of up to three and two years respectively Salary, irrespective of where the appointment is held, will be on the University Lecturers' scale for non-clinical academic and related staff with entry point related to age and London Allowance where appropriate Research expenses up to £5000 will be available, with the ability to bid for higher figures

The primary criterion for selection will be the scientific merit of the ndividual applicant based on his or her record so far and on future potential Account will be taken of the facilities and intellectual environment being offered by the prospective host department

Applicants who were unsuccessful in previous years should not be deterred from applying in 1990 if they are eligible to do so They should, however, make a fresh application on a new application form and arrange for updated statements from referees to be provided

ROYAL SOCIETY LEVERHULME TRUST SENIOR RESEARCH FELLOWSHIPS

The Council of the Royal Society invites applications from scientists n any subject, including mathematics and engineering, for five appointments from 1 October 1990 to Royal Society Leverhulme Trust Senior Research Fellowships The fellowships have been established through the generosity of Leverhulme Trust to provide opportunities for scientists in mid-career to be relieved of all eaching and administrative duties for a period between one academic term and one year, to do full-time research

Applicants must be aged between 35 and 55, hold a Ph D or have equivalent research experience, and hold a substantive post in a British university or polytechnic Employees of Research Councils, or those in Governmental or other research institutions, will not be eligible The fellowship will normally, but need not necessarily, be held at the applicant's own institution, but may be held at any higher educational or research institution, or industrial research organization, in the UK approved by the Council of tne Royal Society

Fellows will continue to be paid by their employers, the fellow's employing institution will be reimbursed for the full salary costs, ncluding USS and ERNI but not including institutional overheads or other costs The applicant's employing institution will be required to show that appropriate arrangements have been made to cover the applicant's teaching and administrative duties, although the fellow may continue with the supervision of postgraduate or postdoctoral esearch students Fellows may apply for research expenses to cover the costs of consumables, etc , grants cannot be made for equipment The Society will consider further requests for additional grants for travel to conferences or overseas laboratories

RESEARCH FELLOWSHIPS

The Council of the Royal Society invites applications for three appointments, two **EPA Cephalosporin Fund Research Fellowships** and one **Pickering Research Fellowship**, in the areas of medical, biological or chemical sciences, at least one of which, the Pickering Research Fellowship, will be made in the area of botany or chemistry

The Head Bequest Committee of the Royal Society invites applications for the **Henry Head Research Fellowship** for original research in neurology in its widest aspects

Conditions of tenure

All the appointments are subject to the Society's General Regulations Governing Royal Society Research Appointments All candidates must have a Ph D or equivalent research experience and there are no restrictions on nationality or place of tenure

Tenure for all appointments is three years in the first instance from 1 October 1990 and renewable for a further two years Superannuation benefits will be provided and research expenses up to £5000 will be available, with the ability to bid for higher figures The point of entry on the salary scale will be determined by Council or the Head Bequest Committee

Salary

Salary scale (for all appointments) £16 207 x £669 to £18 883 x £657 to £20 854 x £705 to £23 674 per annum

Age limit Over 33 and preferably below 40 on 1 October 1990

ROYAL SOCIETY–BOC LIMITED RESEARCH FELLOWSHIP

Applications are invited by the Council of the Royal Society for the Royal Society–BOC Limited Research Fellowship for independent and original scientific, technological or engineering research in a field of direct interest to BOC Limited BOC Limited is a wholly-owned subsidiary of the BOC Limited Group plc, which is an international company manufacturing and marketing a range of products including gases and related products, it has additional interests in health care, high vacuum technology, carbon and carbide products and distribution services The successful candidate will be expected to work on gases and related products which are the specific responsibility of BOC Limited, and will be expected to liaise closely with BOC Limited It is expected that applicants will establish a dialogue with the appropriate BOC Limited department before submitting their applications

Candidates must be citizens of a member country of the European Community and must hold the fellowship in a university or polytechnic department in the United Kingdom Applicants should preferably be under 33 on 1 October 1990

The appointment will be tenable for three years from 1 October 1990 and may exceptionally be renewable for a further two years, such renewal being considered at the end of the second year of tenure The stipend scale will be £15 538 x £669 to £18 883 per annum, and the point of entry will be determined by Council Superannuation benefits will be provided and, where appropriate, London Allowance Some provision for research expenses will be available

RUTHERFORD SCHOLARSHIP

This award, for graduates of exceptional promise and ability who are under 26 years of age on 1 May 1990, is for research in any branch of the natural sciences, with a preference for experimental physics if there are candidates of similar merit Two appointments will be tenable for three years, from 1 October 1990 or such other date as may be arranged, in some part of the British Commonwealth other than that in which the applicant graduated The value, adjusted to meet the circumstances, will be £5650 per annum on appointment if held in the United Kingdom, with allowances for travel, contribution to university fees, etc It will not normally be awarded to a graduate who has already held a senior research award

or all appointments apply (by letter) for application form and further details (*stating for which appointment*)

Address Mrs U M A Tokle, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG

Closing date 16 February 1990

No applications forms will be issued after 2 February 1990 and no applications arriving after 4 p m on 16 February will be considered

(2114)E

Yale UNIVERSITY

The McKnight Foundation

Molecular Genetics of the Flowering Process

Graduate and Postdoctoral Fellowships

An interdisciplinary research program at Yale University sponsored by The McKnight Foundation

Applications are invited for Graduate and Postdoctoral Fellowships to join an interdisciplinary research program undertaken by ten faculty members in three departments at Yale University (Biology, Molecular Biophysics & Biochemistry, and Forestry & Environmental Studies)

Ongoing projects include the following

- genetic analysis of cell-cell interactions in floral structures
- mutational and molecular fine-structure analysis of floral genes
- ectopic expression of growth regulators in the flowering process

The training program also includes technical workshops, seminar programs, and extensive collaborative work. Graduate applicants must apply to the Director of Graduate Studies, Department of Biology, P O Box 6666, Yale University, New Haven, CT 06511 U S A, Telephone (203) 432-3538 by January 3, 1990 Postdoctoral applicants should apply directly to the Project Coordinator with CV, three letters of reference, and a research interest statement Postdoctoral fellows will be encouraged to develop independent collaborative projects on flowering

For further information, contact Prof. Ian Sussex (Project Coordinator), Yale Biology Department, P.O. Box 6666, New Haven, CT 06511 U.S.A.

Yale University is an Affirmative Action/Equal Opportunity Employer

(NW4576)E

HELLENIC PASTEUR INSTITUTE

POSTDOCTORAL FELLOWSHIP IN BIOCHEMISTRY

A postdoctoral position (2-3 years) is available for a young Biochemist or Biologist, to join a team studying membrane proteins, using biochemical approaches

Applications, including a C V and names of referees should be sent to Dr K Soteriadou, Department of Biochemistry, Hellenic Pasteur Institute, 127, Vas Sofias Ave, Athens 11521, Greece Fax 1-6423498 Tel 1-6430044 (W6715)E

The Queen's University of Belfast

RESEARCH FELLOWSHIP

Division of Biochemistry

Applications are invited from suitably qualified EC citizens, postdoctoral or postgraduate, for a fellowship to work with Dr R J H Davies on the photomodification of DNA, emphasising laser-induced processes and sequence-directed photosensitisation The research is part of a collaborative programme, funded by the EC, with groups at Trinity College, Dublin (Professor J M Kelly and D J McConnell) and the Museum National d'Histoire Naturelle, Paris (Professor C Helene and Dr T LeDoan) Generous travel funds are available for consultation and secondments between the participating laboratories The fellowship is available from 1 February 1990, or an agreed date thereafter for one year, with renewal by mutual agreement

Salary range £10,458–£14,169 per annum, placing depending on age, qualifications and experience with superannuation under USS

Informal enquiries may be made from Dr Davies, tel Belfast (0232) 329241 ext 2701

Applicants, quoting ref 89/R2, should submit a curriculum vitae, including the names and addresses of two referees, to the **Personnel Officer, The Queen's University of Belfast, Northern Ireland, BT7 1NN** Closing date 8 December 1989 *The University is an Equal Opportunity Employer* (2080)E

UNIVERSITY OF CANTERBURY

New Zealand

POSTDOCTORAL FELLOWSHIPS (CHEMISTRY, GEOGRAPHY, ZOOLOGY)

Applications are invited for three two-year University Grants Committee Post-Doctoral Fellowships

Chemistry

Test methods for aluminium toxicity in soils The Research Fellow will be expected to participate in an ongoing project on the development of field tests for the toxic fraction of aluminium soils (Ref C89/01) Enquiries may be made direct to Dr H K J Powell, Department of Chemistry

Geography

Climatic change, sea-level rise and hazards to coastal land, as these matters relate to ongoing research in the Department of Geography (Ref C89/02) Enquiries may be made direct to Dr R M Kirk, Department of Geography

Zoology

Salmon stock development The Fellow is expected to be competent in recombinant DNA technology (Ref C89/04) Enquiries may be made direct to Dr F Y T Sin, Department of Zoology

Applicants must be aged 35 years or less and have been awarded, or have qualified for the award of, the degree of Doctor of Philosophy, or a degree equivalent thereto, or have submitted a thesis for examination The age limit may be relaxed in special circumstances or in order to avoid hardship to an applicant for a Fellowship

The emolument for a Fellowship shall be at a rate not exceeding the lowest step of the Lecturer salary scale (NZ$36,000 per annum)

Before submitting a formal written application, candidates are requested to obtain further particulars and Conditions of Appointment from the Appointments Officer, Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF

Applications quoting the appropriate reference code close with **A W Hayward, Registrar, University of Canterbury, Private Bag, Christchurch, New Zealand,** on 14 December 1989 (W6723)E

WELLCOME FELLOWSHIPS IN MEDICAL SCIENCE

Wellcome Trust Senior Research Fellowships in Medical Science in Australia

APPLICATIONS FOR 1991

The Trustees invite applications for two five year senior research fellowships to be held in biomedical science in Australia These fellowships, to commence in 1991, continue the scheme which has provided funding for twelve outstanding Australian biomedical researchers since 1984

Australian nationals who are either medically qualified or science graduates, probably aged between 30 and 37, who wish to make their career in Australia and undertake research in an Australian academic institution are eligible to apply whether or not they are currently working in Australia Direct personal enquiries by possible candidates are welcome but formal applications must be submitted by the Head of Department in the University where the candidate will work

The salary offered will be according to age and experience and on appropriate Australian academic scales with up to A$20,000 per annum ancillary support

Nominations must be submitted on the appropriate application form Closing date for the award is 31 January 1990 For information and application forms please contact

Dr Peter Castaldi
The Royal Australian College of Physicians
145 Macquarie Street
SYDNEY NSW 2000 AUSTRALIA

Telephone (02) 247 4461 Fax (02) 231 3120

(W6724)E

continued on page

ASSISTANTSHIPS

BIOPHYSICAL SOCIETY ANNUAL MEETING BALTIMORE INNER HARBOR, Feb. 18-22, 1990

SYMPOSIA ON Protein Folding, Protein Structure & Design, Ion Channels, Cell Signalling, Nucleic Acid Structure, Protein Import/Export, Energy Transducing Membranes, Membrane Structure and Dynamics, Excitation-Contraction Coupling, Hormone Secretion, Nuclear Cytoplasmic Exchange

For information Call or Write **Emily Gray, Biophysical Society Office, 9650 Rockville Pike, Bethesda, Maryland 20817 Phn** (301) 530-7114 (NW4577)P

KING'S COLLEGE LONDON

Division of Biomedical Sciences

Postdoctoral or Postgraduate Research Assistantship

Cell Cycle Control

Applications are invited for a 3 year postdoctoral or postgraduate research assistantship to study cytoplasmic factors controlling the initiation of DNA synthesis in vertebrate cells The position, funded by the Cancer Research Campaign, is available from January 1990 Experience in biochemical or molecular aspects of cell biology would be an advantage

Send CV and the names, addresses and telephone numbers of two referees to **Dr R F Brooks, Anatomy and Human Biology Group, Division of Biomedical Sciences, King's College, Strand, London WC2R 2LS** (tel 01-873 2086, Fax 01-836 1799), from whom further details may be obtained

Closing date 7th December 1989 (2110)P

MISCELLANEOUS

RECEPTOR protein produces
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KING'S COLLEGE LONDON

(University of London)

GRADUATE RESEARCH ASSISTANTSHIP IN SYNTHETIC ORGANIC CHEMISTRY

A research assistant is required to work in collaboration with Professor C B Reese on the synthesis of compounds with potential anti-HIV and other antiviral activity

Applicants should hold First or Second Class Honours Degrees from UK universities (or CNAA) or GRSC of equivalent standing, and should have had practical experience of synthetic organic chemistry The post is available now and is tenable for up to three years Salary range £11,466–£18,315 pa including London Allowance with starting point according to age and experience, superannuation benefits are payable Applications together with a curriculum vitae and the names of two referees should be sent as soon as possible to Professor C B Reese FRS, Department of Chemistry, King's College London, Strand, London, WC2R 2LS (2078)P

AWARDS

THE CIBA-GEIGY PRIZE FOR RESEARCH IN ANIMAL HEALTH

The Animal Health Subdivision
of CIBA-GEIGY Limited,
Basel, Switzerland,
is pleased to announce that its Prize
for 1989 will be awarded to

Tilahun D Yilma, DVM PhD
of the
Department of Veterinary Microbiology
and Immunology,
School of Veterinary Medicine,
University of California,
Davis, USA

for his work on the
Development of an Infectious Vaccinia Virus
Recombinant Vaccine for Rinderpest.

The Prizegiving will take place in Basel
on February 7, 1990

(W6717)N 33283

GRANTS & SCHOLARSHIPS

BRITISH LUNG FOUNDATION RESEARCH GRANTS

Applicants are invited to apply for the following categories of awards

1 **Fellowships**
Funds are available to cover the cost of the salary of an individual research worker at the appropriate level for a stated period with an allowance for necessary supporting costs

2 **Project Grant**
Funds are available up to a value of £45,000 to cover any costs appropriate to a stated project This may include salary costs Applications will be considered for awards well below the maximum

3 **Equipment Grants**
Grants will be awarded to the cost of equipment up to a maximum value of £20,000

Awards are available for clinical and non-clinical applicants

Closing dates for applications 20th January 1990

Application forms are available from

BRITISH LUNG FOUNDATION
Kingsmead House, 250 Kings Road, London SW3 5UE
Tel 01-356 5735 (2111)H

continued on page 20

AWARDS

Australian Institute of Marine Science

POSTDOCTORAL AWARDS

The Australian Institute of Marine Science, an Australian Government Statutory Authority, is Australia's premier marine science research institute Its functions are to carry out research in marine science, to assist other persons and institutions engaged in research in this field and to disseminate information relating to marine science

The Institute buildings are located at Cape Ferguson, a coastal site 50 km from Townsville, and adjacent to the central section of the Great Barrier Reef

Up to three Postdoctoral Awards, each of 2 years, are granted each year by the AIMS Council, for research by scientists in the early stages of their postdoctoral career, who are of exceptional promise and have shown proven capacity for original work in marine science research

AIMS conducts its research within four (4) major research programs in an overall plan covering 1986-1991 These programs are Coastal Processes and Resources, Reef Studies, Environmental Studies and Biotechnology and Marine Systems Analysis and Oceanography A total staff of 130 including 29 PhD's is employed at AIMS in these programs The Institute provides excellent support including research funding, but specific requirements should be indentified by applicants for these awards A recently installed Bruker 300 MHz NMR Spectrometer is available for research applications

STIPEND Salary of A$31,525 per annum plus a district allowance of A$890 or A$460 per annum according to dependency

CONDITIONS Relocation costs including economy class airfare to Townsville for an awardee and dependant family and up to A$800 towards removal costs for personal effects are allowed Recreation and sick leave entitlements are the same as those applicable to AIMS employees

APPLICATIONS Persons wishing to apply for these awards should submit a complete **curriculum vitae,** a 2-3 page outline of a projected investigation and support requirements to the Secretary Australian Institute of Marine Science PMB No 3, Townsville MC, Queensland 4810, Australia You should also arrange for reports from three professional referees to be sent to the same address Applicants are encouraged to seek additional information on the Institute and its programs by telephoning the Director (077) 78 9211

Applications and referee reports should be received no later than 3 January 1990 for awards tenable normally not later than 1 May 1990

(W6726)N AR1 89 15 41057

GRANTS & SCHOLARSHIPS continued

ST JOHN'S COLLEGE, CAMBRIDGE

Research Studentships, 1990

The College invites applications for up to seven Research Studentships from men and women who are not already members of the College and who propose to register as Ph D students at the University The Studentships will normally be tenable for three years from October 1990 and of such value as will, in addition to the payment of fees, bring the student's total emoluments to £3,875 a year Applicants must have graduated in their first degree with first-class honours under the British system (or the equivalent grades or assessment overseas), or have a strong prospect of graduating at that level in examinations yet to be taken

Further details and application forms can be obtained from the **Tutor for Graduate Affairs (N), St John's College, Cambridge CB2 1TP** Completed applications must be received by 1 March 1990 (2072)H

THE ROYAL SOCIETY

MARINE SCIENCES RESEARCH GRANTS

1990

The Council of the Royal Society invotes applications from qualified research workers of Ph D or equivalent status for personal grants from three funds for marine sciences

- (a) *Browne Research Fund* for research in marine biology at a marine biological laboratory or elsewhere Preference will be given to those who propose to work on purely scientific problems rather than to those whose work would be primarily directed towards an economic end
- (b) *Maurice Hill Research Fund* established in memory of the late Dr M N Hill, F R S, for research in the general fields of physical and chemical oceanography, marine geophysics and geology, either at sea or in laboratories and institutes having adequate facilities
- (c) *Marshall and Orr Bequest* for the support of marine biological research

Grants may be used for periods up to one year, and are made on the understanding that university or other posts held by applicants will meanwhile be kept open for them Recipients are permitted to use grants while receiving salaries from regular appointments

In 1990 the total amounts available to be shared among all successful applicants will be about £8,000 from the Browne Research Fund, £19,000 from the Maurice Hill Research Fund and £24,800 from the Marshall and Orr Bequest, individual grants will not ordinarily be more than £2,500

Applications should be made on forms to be obtained from **Mrs U M A Tokle, The Royal Society, 6 Carlton House Terrace, London, SW1Y 5AG,** and returned by 15 December 1989 (1928)H

FELLOWSHIPS continued

DUBLIN CITY UNIVERSITY

DEPARTMENT OF CLINICAL MEDICINE

POSTDOCTORAL RESEARCH FELLOWSHIP

Applications are invited from candidates with Ph D's in Immunology or Biochemistry for a fellowship to study protein kinase C involvement in regulation of human T cell lymphoma proliferation and differation Experience with immunochemistry and/or molecular biology would be an advantage

Candidates should submit a full *curriculum vitae* together with the names of three referees to **Dr Dermot Kelleher, Department of Clinical Medicine, TCD Medical School Building, St James' Hospital, Dublin 8**

To arrive **not later than Friday, 8th December 1989** (2093)E

INSTITUT PASTEUR PARIS

Post-Doctoral Position

A fellowship is immediatel available to participate in research program supporte by the E E C concerning th establishment of the total nuc leotide sequence of the *Bacillu subtilis* chromosome Exper ience of molecular biolog would be an advantage

Please send CV, list of publi tions and names of reference to **Dr F KUNST, Instit Pasteur, Unité de Biochim Microbienne, 75724 Pa Cedex 15 (France) Phone 45-68-88-09** (W6730)

continued on p

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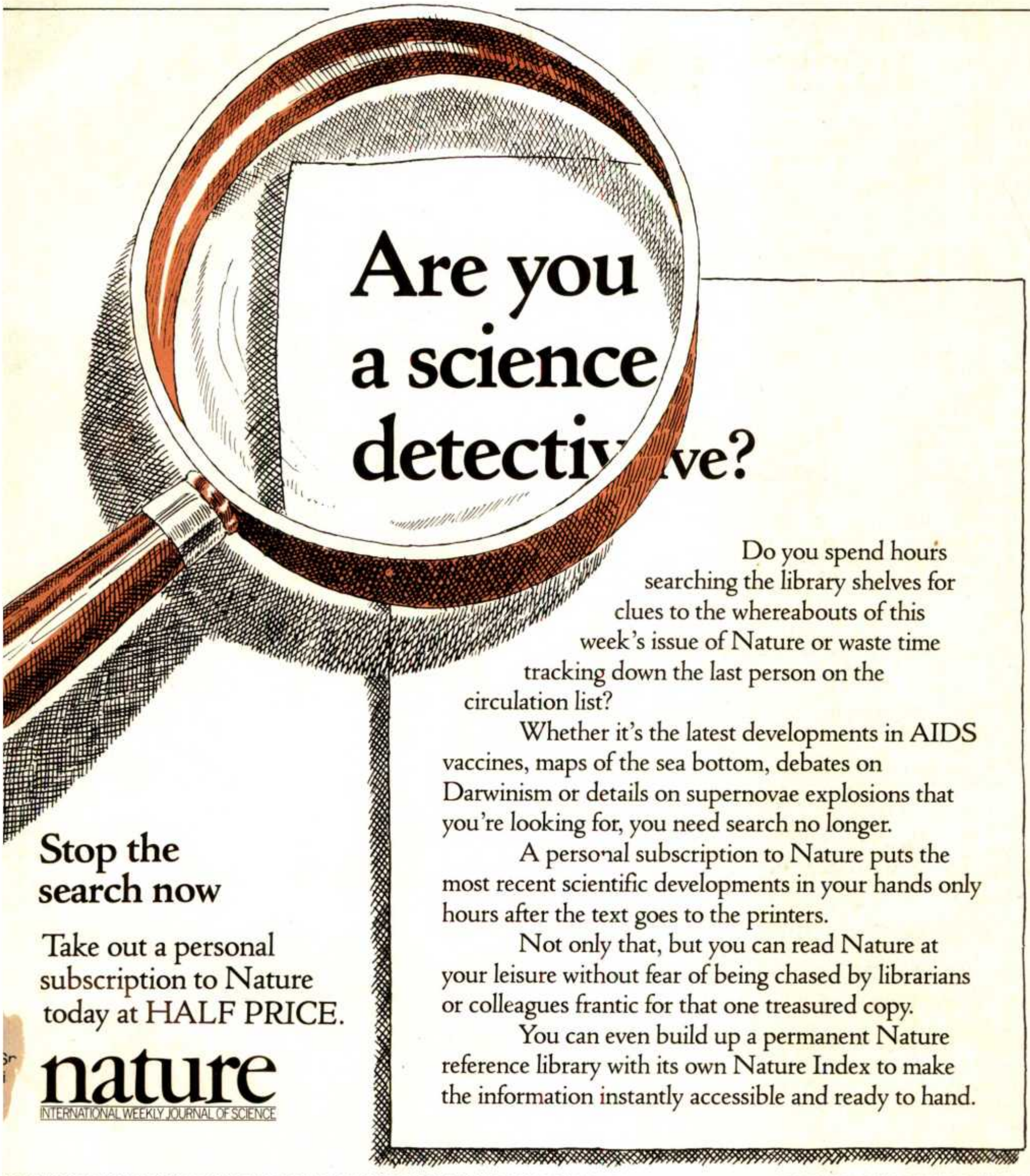

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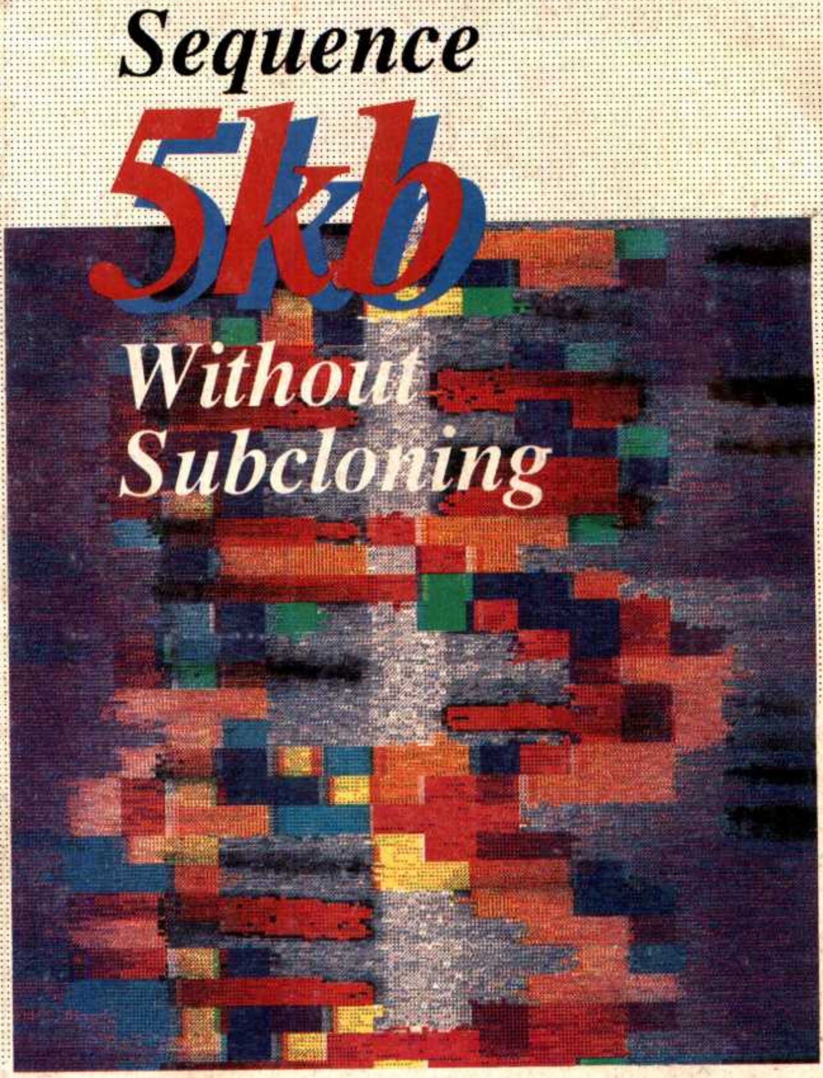

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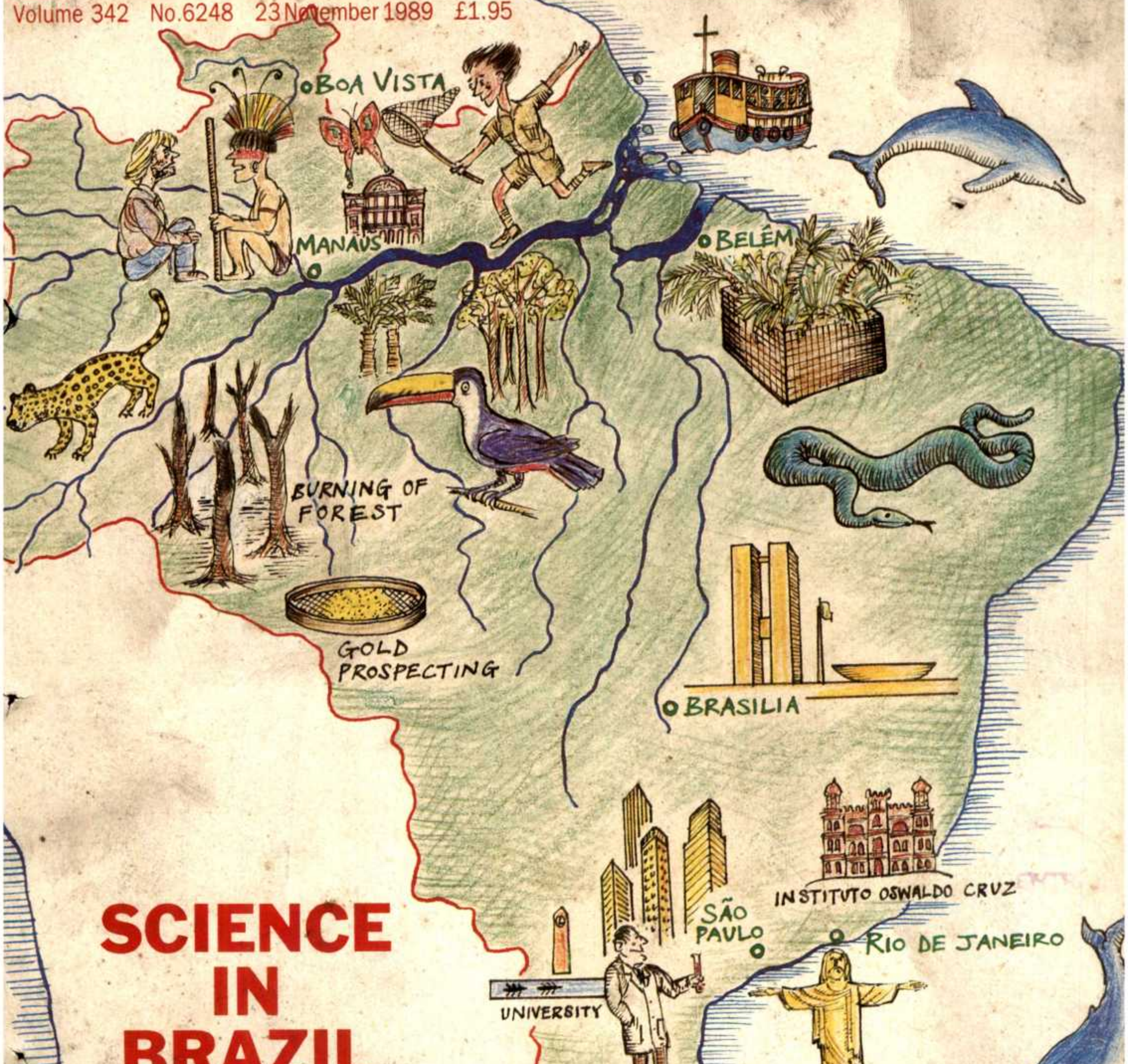

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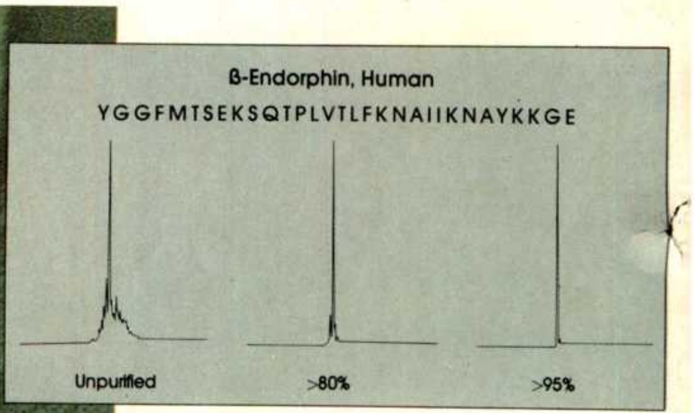

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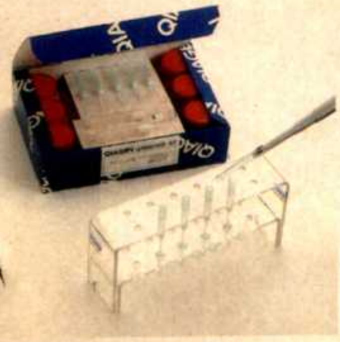

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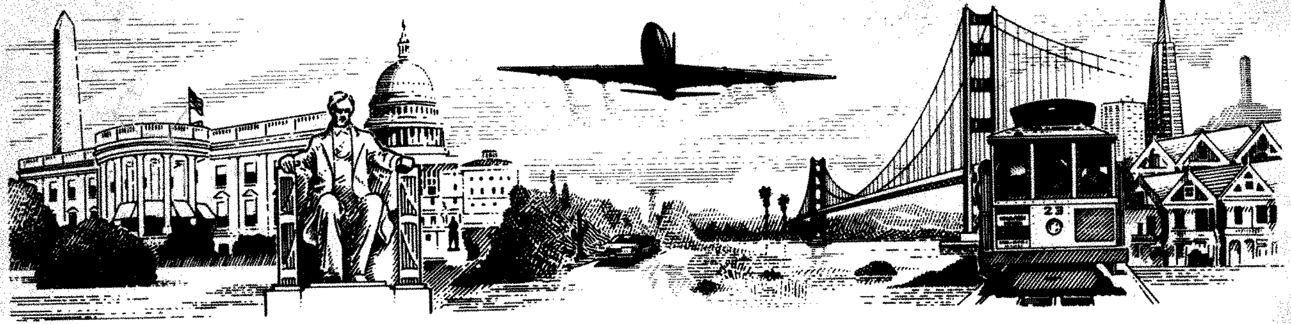
Young Science Writer Awards 1990

WIN A WEEK IN THE USA



Are you aged between 16 and 28? Does the world of scientific discovery fascinate you?

Yes? Then how would you like to spend a week in the USA – at the 1991 American Association for the Advancement of Science Annual Meeting, in Washington D.C.

YOUR WRITING TALENT CAN GET YOU THERE.

We are looking for articles of about 700 words on a scientific discovery or research topic of your choice. It could be anything from astrophysics to animal behaviour or AI to HIV.

You select the field – and then write an article that both informs and entertains specialist and general newspaper readers alike.

THE PRIZES

The competition is divided into two age groups: 16-21 and 22-28, with a total of 12 prizes in each age group:

- The best article will earn its author a trip to the USA in 1991, as well as the satisfaction of seeing the winning piece published in The Daily Telegraph's Monday Science and Technology page. They will also receive an invitation to attend Science 90, the British Association's Annual Meeting, in Swansea.
- Second prize is a year's donated subscription to Nature, and a year's subscription to New Scientist, plus an invitation to Science 90. All runners-up have a chance of seeing their entries published in The Daily Telegraph.
- There are 9 runner-up prizes of a year's donated subscription to Nature, and a year's subscription to New Scientist.
- A further 25 entrants whose articles are of particular note, will be awarded The Daily Telegraph-British Association Certificate of Merit.
- In addition – for every school pupil reaching the final round of judging, a prize will be awarded to their school.

DR. ROGER HIGHFIELD'S COMPETITION GUIDE

General points:

1. Your article should be about 700 words in length, and not more than 800 words.
2. Your article should be clear and informative and understood by the non-specialist newspaper reader. Scientific jargon should either be explained or avoided.
3. If you feel diagrams or illustrations would be helpful, include them in your entry. It doesn't matter how sketchy they are.
4. 16-21 age group entrants and 22-28 age group entrants not engaged in original research: Write about any scientific discovery, for example, a project being pursued locally or perhaps topics discussed recently in scientific publications. Whatever you choose, you must identify your sources.
5. 22-28 age group entrants engaged in original research: It doesn't matter whether your research is complete or not; citing other work is perfectly acceptable if your own has not yet borne fruit.

For a more detailed guide and copies of last year's winning entries, please write to the competition address.

THE JUDGES

Professor Lewis Wolpert: President, British Association for the Advancement of Science. **Sir Walter Bodmer:** Director of Research, Imperial Cancer Research. **Mr. Richard Fifield:** Executive Editor, New Scientist Magazine. **Dr. Peter Newmark:** Nature Magazine. **Professor Sir David Phillips:** Laboratory of Molecular Biophysics, Oxford University. **Professor Heinz Wolff:** Institute of Bio Engineering, Brunel University. **Dr. Roger Highfield:** Science Editor, The Daily Telegraph. **Dr. Mary Archer:** Scientist and Company Director. The judges will assess your articles on content, quality of writing, your age, and any graphic illustrations you may propose.

Send your entry to: Ms. Julie Dallison, British Association, Fortress House, 23 Savile Row, London W1X 1AB, to arrive not later than 24 February 1990. Make sure that your entry clearly shows the category you are entering, your full name, date of birth, school/university/place of work, contact address and telephone number.

COMPETITION RULES

1. Entrants must be aged between 16 and 28 (inclusive) at 24.2.90. 2. Entries must be typed or legibly written in ink or ballpoint. 3. Entries over 800 words in length (not including description of any graphic illustration) will be disqualified. 4. The judges decision is final and no correspondence on the nature of assessment or the results will be entered into. 5. Competition is open to UK residents only. Employees (and their families) of The Daily Telegraph plc, subsidiary or associated companies, advertising and promotion agencies are not eligible. 6. The Daily Telegraph Standard Competition Rules apply. They can be obtained by sending a stamped addressed envelope to Ms. Julie Dallison at the address shown above.

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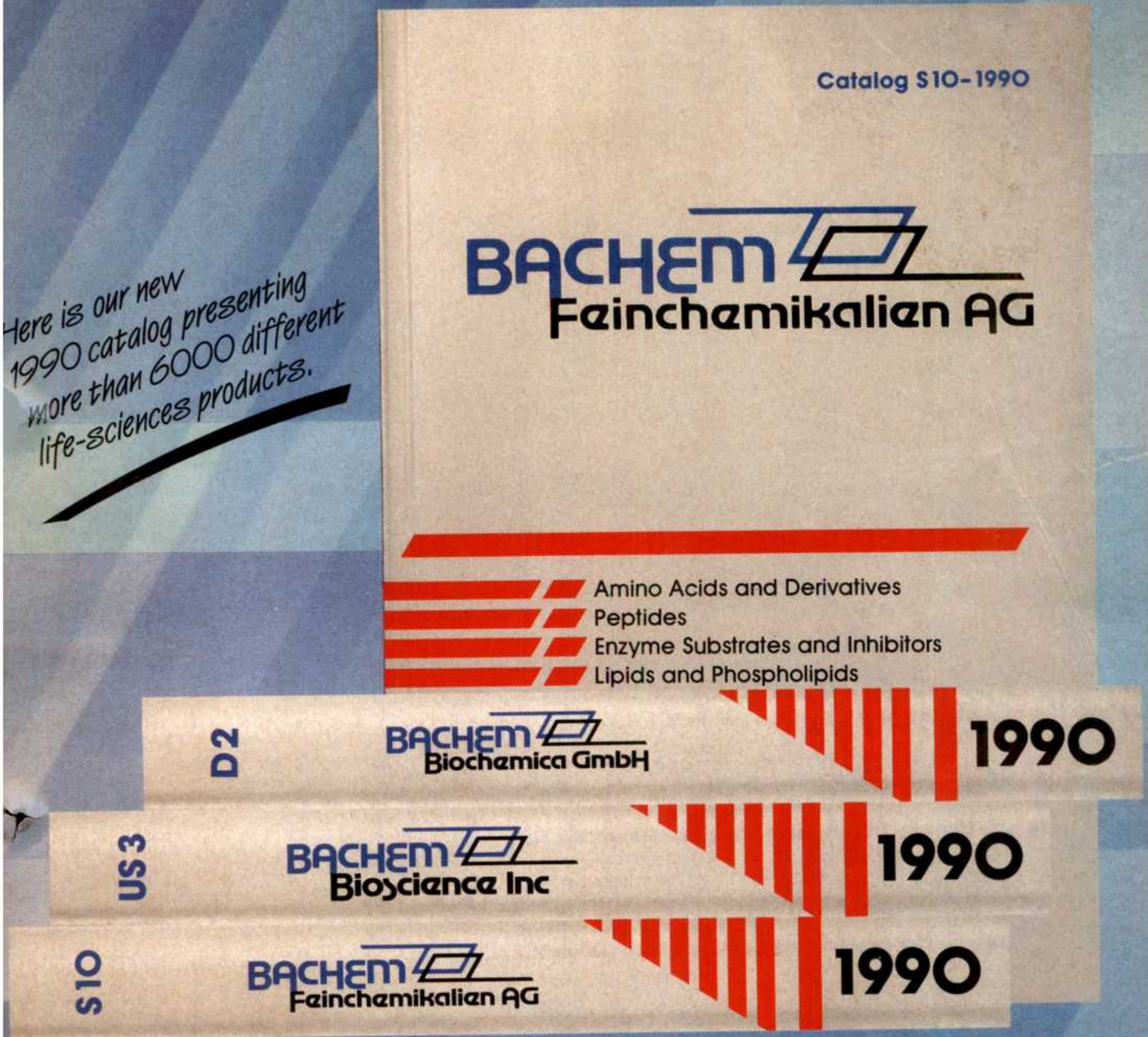

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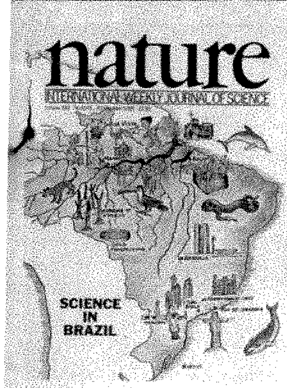

nature

23 November 1989
Vol. 342 Issue no. 6248

◀ 'Science in Brazil', a 20-page feature, begins on page 355. Cover artwork: Andrew Birch. The Amazon region is also the subject of a Letter and News and Views (see panel below) and Scientific Correspondence, page 350.

THIS WEEK

No, definitely not

Exhaustive attempts by workers at the UK Atomic Energy Authority's Harwell Laboratory to reproduce the much-publicized results of Fleischmann *et al.* and Jones *et al.* have failed to support a 'cold fusion' explanation for the phenomena. Spurious effects that might contribute to apparent positive results include noise from neutron counters, cosmic rays, miscalibration of calorimeters and variable electrolytic enrichment of tritium. Page 375.

Breaking-down barriers

Mutations affecting the mismatch DNA repair system are found to increase the frequency of genetic recombination between two bacterial species — *E. coli* and *S. typhimurium* — by up to 1,000-fold, showing that this system is one of the barriers preventing genetic exchange between species. Page 396.

Vital factor

The cloning of human hepatocyte growth factor is reported on page 440. The availability of functionally active recombinant human HGF will be of value in research into liver function, and in the long-term HGF may be useful clinically for enhancing liver regeneration after surgery, and in the diagnosis and treatment of hepatitis.

More making less

Although the link between polar ozone depletion and halocarbon chemistry has been clearly established, attempts to account for the rate of ozone loss in Antarctica in terms of known photochemical processes have had limited success. Additional ozone-depleting mechanisms may therefore be involved, and photolysis of OClO is suggested as a candidate, page 405.

RNzyme specificity

The definition of the precise nature of the interactions between the guanine base of guanosine and the *Tetrahymena* self-splicing intron demonstrates the mechanism by which an RNA enzyme binds specifically to its substrate, page 391.

Ribosome switch

In the malaria parasite *Plasmodium falciparum,* two different ribosomal RNAs predominate at different stages of the life cycle. How the transition is made is investigated on page 438.

Amazonian portents

The Amazon basin contains roughly half the Earth's tropical forest. What would happen to the climate of the region if relentless deforestation were to lead to the replacement of trees with savannah and pasture? A three-year computer simulation provides some of the answers. Pages 411 and 343.

Making of a pathogen

The transfer of a cutinase gene from one fungal species to another confers the ability to infect intact fruit, providing additional evidence that this enzyme is important in enabling plant pathogens to overcome the physical barrier of the plant cuticle. Page 446.

Ear to the ground

The evolution of the middle ear is central to the discussion of how the first tetrapods adapted to life on land, and it seems that the earliest tetrapods may have retained a fish-like breathing mechanism. The morphology of the stapes bone of *Acanthostega*, a primitive tetrapod from the Upper Devonian of Greenland, suggests that it was involved in breathing movements rather than in the functioning of an impedance-matching ear. Pages 425 and 342.

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Upper bounds on 'cold fusion' in electrolytic cells
D E Williams, D J S Findlay, D H Craston, M R Sené, M Bailey, S Croft, B W Hooton,
C P Jones, A R J Kucernak, J A Mason & R I Taylor **375** ▶

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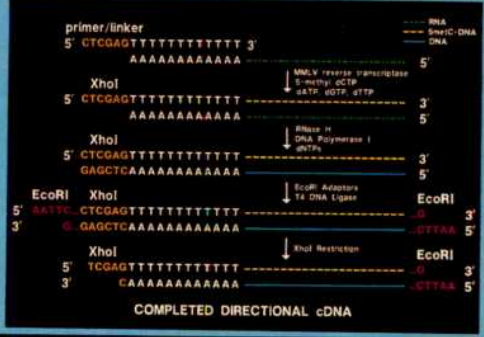

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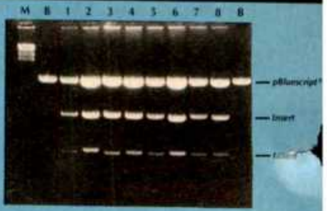

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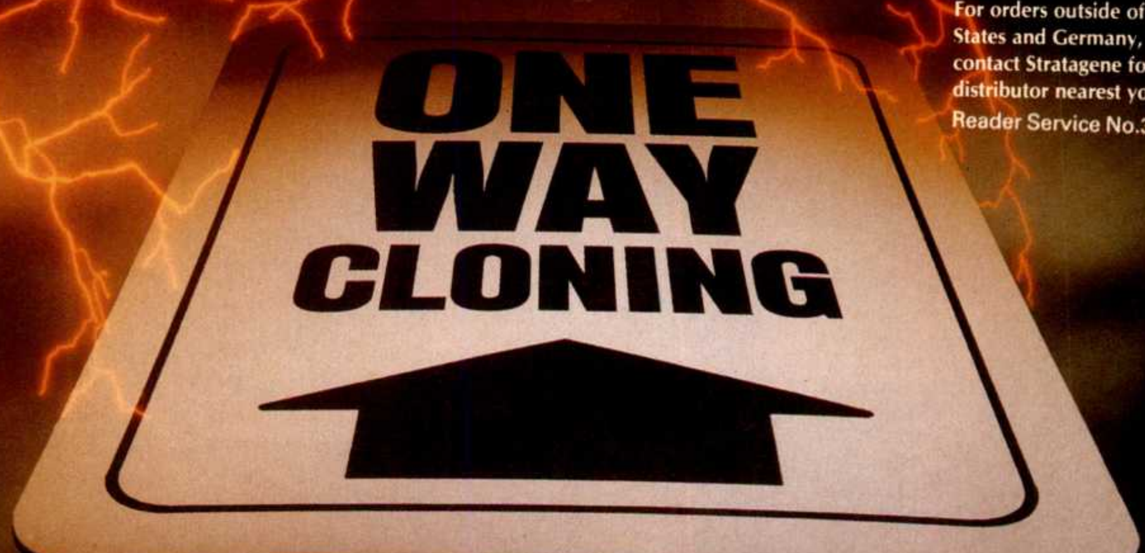

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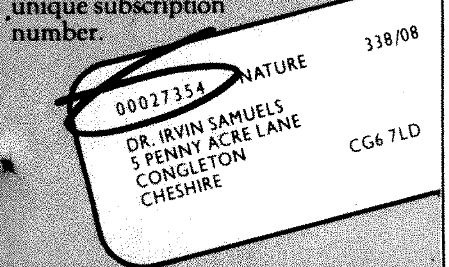

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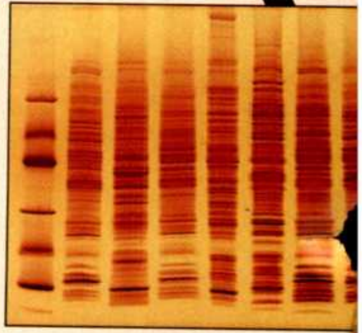

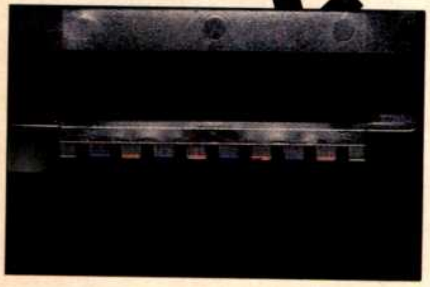

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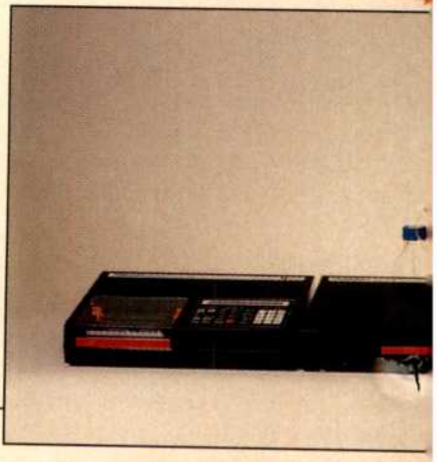

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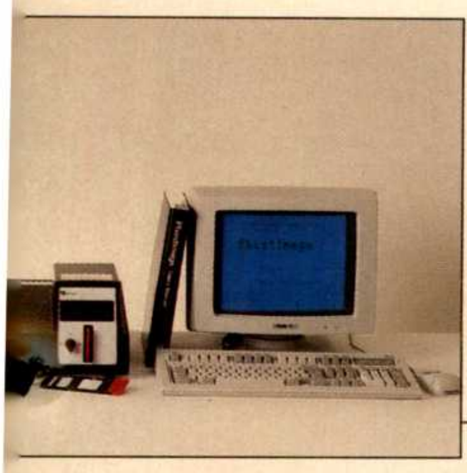

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Eckhard R. Podack, University of Miami
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Molecular Pathogenic Mechanisms

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Diseases of Abnormal Antigen Receptor Formation
Ilan R. Kirsch, NCI-NMOB
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Molecular Mechanisms in Autoimmunity
Lawrence Steinman, Stanford
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Disordered Regulation of Lymphocyte Activation
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Genetic Disorders of Surface Molecules
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Bernard F. Mach, University of Geneva
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Molecular Mechanisms of Immune-Mediated Tissue Injury
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Joan Stein-Streilein, University of Miami
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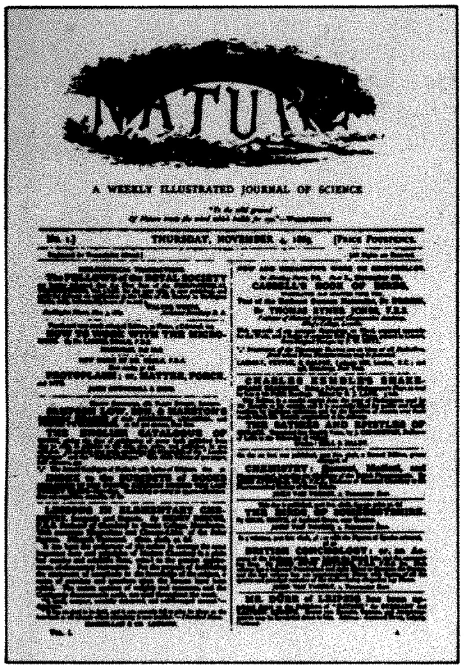

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NATURE VOL 342

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23 NOVEMBER 1989

Moderation of abortion debate

The chairman of the US Republican Party is not the most familiar source of wisdom on abortion, but everybody will benefit from what Mr Lee Attwater has learned in this month's elections in the United States

THE months ahead may see a marked attenuation of the public rows about the practice of legitimate abortion. That, at least, seems to be one of the portents of this month's elections in the United States, when New York elected its first black mayor, when governorships were up for grabs in states such as New Jersey and Virginia and when several candidates for less exalted offices discovered, against expectation, that a strong anti-abortion platform could be an electoral disadvantage. The shift was marked enough for Mr Lee Attwater, the chairman of the Republican National Party proclaim that his party is a tolerant party that welcomes a variety of opinions, even on matters as contentious as abortion. Most of the anti-abortionists disappointed at the polls appear to be Republicans.

That abortion is a contentious issue, and will remain so, requires no explanation. Whatever the resolution of the tediously familiar argument about the point at which human life begins (conception, implantation, the emergence of the neural crest or the like), there is no doubt that the majority of fetuses now aborted are alive by almost any criterion. That is why, where abortion is permitted (in the United States, abortion is the prerogative of state governments, but the Supreme Court could change that), explicit conditions must be satisfied before an abortion can be carried out. The general principle derives from the notion that if, at childbirth, there is a choice between the survival of the mother and of the child, the mother's life takes precedence. To be able to demonstrate, say by amniocentesis, that a fetus is genetically handicapped is not by itself a sufficient cause for legalized abortion, in principle, at least, it is necessary for a woman seeking an abortion also to demonstrate that the birth of such a child will be damaging to her, if not physically then psychologically.

Opponents of abortion are of three kinds: those who call abortion murder whatever the circumstances, those who complain that the conditions that must be satisfied before an abortion may be legally carried out are insufficiently stringent and those who complain that the preconditions are so loosely applied that legal abortion is elective abortion. The arguments for legalized abortion, in similar order of increasing moderation, are that a woman's body (and whatever is inside it) is at her own disposal (the feminist view), that unwanted children are genuinely damaging and that legal but regulated abortion is preferable to illicit practice on a similar scale. In an ideal world, governments would seek to blunt unresolved argument by diminishing the scale of the problem by post-abortion counselling and easier access to birth-control clinics, but these measures are not popular with extremists. Until this month's elections, this seemed to matter to politicians. It may be less important now.

This journal has no brief to grind an axe on the abortion issue proper and does not do so, but there are several related issues that affect what happens in laboratories. In the United States, the Secretary of Health and Human Services has just extended a moratorium on the use of fetal tissue in transplants out of — it now seems — misplaced respect for anti-abortion forces (see *Nature* **342**, 105, 1989). In Britain, the government's bill to regulate embryo research due to be published this week will be a vehicle for a parliamentary battle on abortion. But everywhere even those content with the legislation on abortion that may be in force should be concerned that its extreme opponents ('right-to-life' or 'abortion rights', as the case may be) are implacably opposed. It cannot much benefit grown-up societies that there should be apparently irreconcilable conflicts over the propriety of the law.

Despite a long history of bitter dispute, there is a chance that the two sides will be able to reach an accommodation with each other. That does not require that conflict should vanish overnight, but merely that people who are implacably opposed should learn that the other side has a case, but perhaps not the whole of what the extremists claim. Attwater may not be the most likely source of good sense on abortion, but his plea for tolerance deserves a hearing. ☐

Brain drain in flood

Migrations of skilled Europeans in the past few weeks are only the beginning. Employers should respond

BY the end of 1989, the numbers of people who will have moved permanently from one country to another will be a record for recent times. Even before the East German frontier with the West was opened last week, some 50,000 people had made their way around it in the previous two months. That exceeds, but not by much, the numbers who will have left the Republic of Ireland (mostly for the

United States) in 1989. Almost as a direct consequence, Turkish guest-workers will be displaced from West Germany in numbers comparable with those of the ethnic Turks being expelled from Bulgaria. In present circumstances, ethnic Hungarians in Romania would be heading for Hungary if their government would let them go, but the Soviet Union may end this year having lost 500,000 people, mostly to the United States and Israel. And then there are the boat people from Vietnam, not to mention those who escape from Cambodia while they can.

It is natural to regard these huge migrations as welcome signs of the liberalization of frontiers all over the world, but even voluntary migration is never trouble-free. People who complain of the trauma of moving house within a single country should multiply their impressions by some large number, say ten, to allow for the difficulties of settling into an entirely unfamiliar environment. That is why the populations picking up their own roots to go elsewhere are naturally self-selected to include the most employable, which means those whose skills are technical and thus most portable.

There is worse to come. If Eastern Europe and the Soviet Union now keeps to the spirit of the Helsinki agreements, *perestroika* will have to be made to work to keep the skill it needs where it is needed. But even within the West, the growing conviction that the European Economic Community will yet become a true common market will mean that there are quite unpredictable movements of skilled people between member countries, which will further complicate the general drift of people to North America. What the British call the brain drain will become a general phenomenon.

How can the losers in this new labour market defend themselves? The only simple answer is that they will have to pay market prices in some sense or another. That does not necessarily mean that money salaries must everywhere become identical; working and living conditions are also important. Researchers and academics generally, but also people with skills in electronics and computer usage, are especially vulnerable in present circumstances. Wise employers, governments included, should begin planning now for the structural changes required to succeed in the coming competition for competence. □

Conduct unbecoming

Runners-up to science prizes receive no recognition but should know better than to complain.

ONE of the stipulations in Alfred Nobel's will, finalized a year before his death in 1895, was that the prizes to be awarded annually in his name should be awarded for discoveries made in the preceding year. Almost without exception, this stipulation is disregarded. But another, which limits to a maximum of three the number of people who may share a prize, is still honoured. Dominique Stéhelin, a French virologist, continues to argue (see page 329) that he should have been the third person named for this year's prize in physiology or medicine, awarded to Michael Bishop and Harold Varmus.

As all agree, Stéhelin was the main pair of hands behind the experiments that first showed that the oncogenes of tumour viruses are stolen and corrupted versions of genes from the cells that the viruses infect. So much is acknowledged by the fact that he is the first author on the 1976 *Nature* paper that reported this discovery and which the Nobel assembly cite as the seminal piece of work. Nevertheless, only the head of the laboratory in which Stéhelin was a visiting scientist and the head of an associated research team have been awarded the prize.

Is this rough justice? With the absolute rule that all Nobel decisions are final and without appeal, it will be 50 years before the reasoning of the Nobel committees can be examined. It seems likely, however, that two questions that will have been asked are these: would Stéhelin, had he been working in any other laboratory, have been an author on the seminal paper, and would the paper have emerged from the Bishop/Varmus milieu even had Stéhelin not been there? It is likely that the answers were no and yes, respectively, considerably diminishing Stéhelin's case.

Nonetheless, there is no rule to stop the Nobel authorities from including Stéhelin had they so wished. Precedent was clearly against it, with the exclusion of Jocelyn Bell from the 1974 physics prize although it was she who spotted the pulsar for which, in particular, Antony Hewish was awarded his half of the prize, as a notable example. On the other hand, Georges Kohler, the visiting scientist who worked on the discovery of monoclonal antibody technology in César Milstein's laboratory, shared the 1984 prize for physiology or medicine. It is, of course, up to those who award prizes to set the rules but in the case of the relative merits of dirty hands to fertile minds there seems not to be one.

Stéhelin's immediate reaction was ungentlemanly. His claims can surely not have been overlooked. There is no doubt that it is harder to achieve international visibility in Lille than in San Francisco, but Stéhelin, who shared with Sydney Brenner and Walter Gehring the valuable and esteemed 1987 Jeantet prize for European biomedical scientists, was certainly in view from Stockholm.

But how wise is his new outburst, which seems largely to have been occasioned by some remarks by a member of the committee that recommended the winners? If the press has accurately reported these remarks, Stéhelin has some cause for complaint, but his response will do him no good. Even so, his contributions should be generously acknowledged at the prize-giving ceremonies in Stockholm next month. That will also be a time for the Nobel authorities to reconsider the terms of the prizes. With teamwork now the rule rather than the exception in research, for how long can they continue to award their prizes on the basis that the winners take all, and the runners-up receive no recognition? □

COBE starts its search for galactic fingerprints

- Satellite launched after four-year delay
- Looking for cosmic microwave irregularities

Washington

THE launch on 18 November, of the COBE (Cosmic Background Explorer) satellite from Vandenberg Air Force Base in California marks the start of a long-awaited astrophysical mission that had been delayed almost four years after the explosion of the space shuttle Challenger.

COBE's principal job is to look for irregularities in the cosmic microwave and far infrared backgrounds, an urgent task for cosmologists but one which has mostly passed beyond the capabilities of ground-based or balloon-flight experiments.

COBE was due to go on a shuttle flight soon after the Challenger disaster of January 1986, but NASA (National Aeronautics and Space Administration) decided shortly thereafter to switch it to a *Delta* rocket. This necessitated dismantling the finished satellite and putting the same instrumentation into a much smaller package. The reduction was achieved by omitting on-board thrusters, which would have been needed to propel COBE into its 900-km high, approximately polar orbit from the low shuttle orbit. The Delta launcher can put COBE directly into place.

The $160-million COBE satellite carries three instruments, and will scan the entire sky twice during it's one-year mission. By virtue of its polar orbit, COBE looks always at right angles to the direction of the Sun, whose light it must avoid to prevent damage to the detectors and interference with the measurements.

The two conical antennae of the Differential Microwave Radiometer will measure the difference in intensity of the cosmic microwave background, at three different frequencies, between two slightly displaced spots in the sky. This will yield an all-sky map of the variation of the microwave background from its average temperature of 2.7 K.

Temperature variations at some level are predicted by all theories of galaxy formation, the primordial variations in the cosmological density which acted as the 'seeds' of modern galaxies must also have left their mark as small irregularities in the microwave background.

Over the past two decades, experiments done from the ground, balloons and short-flight rockets have produced no conclusive detection of temperature variations. When the earliest experiments were done, simple galaxy formation theories predicted temperature variations of as much as one part in a thousand, but as increasingly sophisticated instruments have brought the upper limit down to as little as a few parts in one hundred thousand, galaxy formation theories have been of necessity refined and adjusted to creep under the lowering wire of the measurements. There is a general feeling now that theory has few recourses left, and that if COBE does not find any temperature variations at a level perhaps an order magnitude less than the present best observations, then revision of standard cosmological ideas may be unavoidable.

The job of another of COBE's instruments, the Far Infrared Absolute Spectrophotometer, has become more crucial during the enforced delay of the satellite's launch. Last year, researchers from Nagoya University and the University of California at Berkeley published measurements obtained by a rocket-borne detector which indicated a substantial excess in the background intensity at wavelengths between 1.0 and 0.1 mm, above the peak frequency of the microwave background, but below infrared emission from interstellar dust. The Nagoya–Berkeley team saw emission in this far infrared region an order of magnitude more intense than they expected, no convincing theoretical source has yet been identified, and some cosmologists believe that the detection is the result of some systematic instrumental error. COBE's far infrared detector should confirm or invalidate the Nagoya–Berkeley measurements, in either case telling cosmologists something about a region of the spectrum which, because of atmospheric opacity, has been little studied so far.

COBE's third detector, the Diffuse Infrared Background Experiment, goes to even longer wavelengths, and will look for emission which could be the accumulated radiation from distant, early galaxies.

By scanning the sky over a frequency range from microwave to infrared, COBE is in effect scanning the process of galaxy formation, from the imprint of density fluctuations retained by the microwave background to the faint, red-shifted emission reaching us now from the first generation of protogalaxies. Cosmologists are guaranteed to get something to think about, especially from the microwave experiment: if COBE finds the long-sought irregularities, they will at last have some numbers to put into their theories, if it finds nothing, they will need some new theories.

David Lindley

PRIZES

Nobel dispute continues

London

DOMINIQUE Stéhelin, the French scientist who claims he should have shared this year's Nobel prize in physiology or medicine, has expounded his case in an open letter to the committee that recommended the winners, Michael Bishop and Harold Varmus. But the committee has no intention of responding.

In his letter, Stéhelin amplifies his view that it is unjust to have excluded from the prize the person who carried out the crucial experiments for the key discovery cited in the announcements of the awards. He argues that his exclusion is strongly detrimental because the "prize carries an unparalleled aura and prestige, lending it absolute and unquestioned authority in scientific quarters and the eyes of the public", and requests that the committee finds a way to repair the damage that has been done.

Apart from the fact that his name does not even appear in the documents that accompanied the announcement, most of Stéhelin's complaints are directed at comments made by one member of the committee in response to Stéhelin's initial outburst. Erling Norrby was quoted in newspapers as saying that the French researcher had not published anything of interest since 1976, when the key paper by Stéhelin, Varmus, Bishop and Peter Vogt was published in *Nature*, and that whereas Bishop and Varmus's contributions had already been recognized by several prize awards, Stéhelin had none.

Stéhelin, who directs a unit within the Institut Pasteur in Lille, responds that among the more than 100 papers he has published since 1976 are reports of the discovery of six new oncogenes, and that he has been awarded four prizes, among which is the 1987 Jeantet prize for European biomedical research, which he shared with Sydney Brenner and Walter Gehring.

Jan Lindsten, secretary of the Nobel committee that recommended Varmus and Bishop, says that Norrby was speaking in a personal capacity, and that the committee will not be responding to Stéhelin. It is, he said, increasingly difficult to select the appropriate winners as research is increasingly a collaborative affair. While pointing out that the committee is empowered to consider only those who have been nominated, he refused to say whether there were any nominations for Stéhelin. Lindsten emphasized that the prize was given for more than the 1976 paper alone and that the parts played by Stéhelin and many others had been considered by the committee.

Peter Newmark

DATABASES

Johns Hopkins as international host

Washington

With funds from the Howard Hughes Institute (HHI), researchers at Johns Hopkins University (JHU) in Baltimore, Maryland, are planning an international network of databases to support the mapping and sequencing of the human genome. The selection of JHU as host to the first database in the network puts a question mark over the future of the human genome mapping library in New Haven, Connecticut, which is directed by Frank Ruddle of Yale University, and has been supported by the HHI since 1985. But Purnell Choppin, president of HHI, said that researchers at JHU are also seeking federal funds and if they are successful the institute will not fund the database next year.

The new database at JHU, formally named the Genome Data Base (GDB), will provide access to primary data and literature citations, and daily updates of information generated by scientific workshops, the database's scientific editors and individual laboratories.

Users will also have access to OMIM, the on-line version of Mendelian Inheritance in Man, Victor McKusick's classic encyclopaedia of human genetic diseases. Peter Pearson, who worked at the University of Leiden in the Netherlands before he was hired by the HHI to be scientific director of the database, says that he was given a choice between Yale and JHU as the home of a new, more modern database, and that access to OMIM was one of the reasons he chose JHU. With access to both GDB and OMIM, users "will certainly get more for their money", he said. Other databases with which Pearson also hopes to forge links include the mouse genome database at Jackson Laboratory in Bar Harbor, Maine, and that at the Centre d'Etude du Polymorphisme Humain in Paris.

The second centre in the network will be created in London, with the collaboration of the Imperial Cancer Research Fund. Data from the GDB will be transferred at the international human gene mapping workshop in the United Kingdom, and at every subsequent workshop the data will be updated and transferred to a new centre. Eventually there will be an international network of human genome databases.

Kenneth Kidd of the school of medicine at Yale University, a consultant to the Yale database, says that it is trying to obtain funds from other sources, including the federal government, to replace the support from the HHI which this year was about $1.2 million. The university was rejected as a candidate to host the new database amid complaints that the database there is in an outdated format, difficult to access and non-portable. **Christine McGourty**

NUCLEAR POWER

Japan presses on regardless

Tokyo

In spite of the growing anti-nuclear movement and the changing political environments, Japan is maintaining its commitment to expand nuclear power as its primary energy source, according to this year's white paper (policy document) on atomic energy, released last week by the Atomic Energy Commission (AEC).

Japan now has 37 commercial nuclear power plants in operation, supplying nearly 30 per cent of its electricity, and in its annual report two years ago the AEC outlined ambitious plans to increase that figure to 40 per cent by the year 2000. But in the latest report, such optimistic projections are conspicuously absent. The AEC notes that nuclear power has suffered setbacks outside Japan: in the United States, no new plants have been brought into operation for over a decade, West Germany has cancelled plans to build a nuclear-fuel reprocessing plant, and Sweden, Austria and Italy have voted against nuclear power.

Nevertheless, the Japanese report concludes that nuclear power is still "steadily expanding" worldwide and will remain central to Japan's energy policy.

But within Japan, opposition to nuclear power is growing (see *Nature* **333**, 199, 1988). In the past 18 months, the government and power industry has poured tens of millions of dollars into advertising campaigns, lectures and a telephone service to try to win back public support for nuclear power. But surveys earlier this year show that opponents of nuclear power outnumber supporters two to one (see *Nature* **337**, 494, 1989).

The government faces two severe tests of its nuclear power policy in the near future. A huge commercial complex to enrich uranium, reprocess spent nuclear fuel, and store low-level radioactive waste is scheduled to begin operations in 1991 in Aomori Prefecture, on the northern tip of the main island of Japan, but local farmers vehemently oppose the plan.

And in 1992, Japan hopes to start taking shipments of plutonium from Britain and France. The plutonium is derived from spent nuclear fuel from Japanese reactors, and is intended for use in advanced thermal reactors, Japan's first prototype fast-breeder reactor should become operational in 1992. But plans to ship the plutonium by air have already been abandoned because of opposition in the US Congress.

The AEC and the Science and Technology Agency (STA), which oversees Japan's nuclear power research and development, want to construct a new ship, to be operated by the Maritime Safety Agency, for plutonium transportation. But some members of the ruling Liberal Democratic Party (LDP) insist that existing ships of the Maritime Self-Defense Force should be used. However, despatch of these ships overseas is likely to cause public controversy, as it may violate military restrictions in Japan's post-war constitution. In addition, Japan has to face up to the problem of decommissioning nuclear reactors. Throughout the AEC report, nuclear power is referred to as "inexpensive" and "economical".

But the UK Central Electricity Generating Board, which has in the past promoted the low costs of nuclear power, had to admit at a recent public inquiry that the costs of decommissioning and waste disposal make nuclear power more expensive than coal power. This admission weighed heavily in the British decision two weeks ago to abandon plans to privatize the nuclear sector of the power industry (see *Nature* **342**, 213, 1989).

However, British reactors are bulkier, older and more inefficient than their Japanese equivalents. Yukiko Araki, deputy director of STA's Office of Atomic Energy Policy Research, said at a briefing on the AEC report that decommissioning and waste disposal are expected to increase the cost of electricity from nuclear power plants in Japan only to ¥10 (7 cents) per kilowatt hour from the present rate of ¥9. The power industry recently established a fund derived from utility charges to cover the extra costs.

But finding a site for disposal of Japan's high-level waste is another headache for the government. Early plans for deep-sea disposal were abandoned because of objections from neighbouring Pacific island nations. A borehole survey of a site on Hokkaido, Japan's northern island, has been carried out and, says Araki, the government plans to bury high-level waste there at a depth of hundreds of metres. But Hokkaido residents oppose the plan and finding alternative sites will not be easy, especially because of the prevalence of volcanoes and earthquakes throughout Japan.

Finally, the recent rise in strength of the Japan Socialist Party (JSP), following a series of money and sex scandals that have forced the resignation of two prime ministers this year, has added a new political force to the fight against nuclear power.

The JSP has traditionally advocated the abolition of nuclear power, but now takes the more pragmatic position that it will not expand the industry if it gains power in the imminent general election.

Araki says that people in the industry do not now expect Japan to reach the commission's target of 40 per cent nuclear-power-derived electricity by 2000, and that the Ministry of International Trade and Industry is working on new targets. **David Swinbanks**

EARTHQUAKE AWARENESS

Not just in California . . .

Washington

THE biggest earthquake of historical record in the United States occurred not on the West Coast but near the town of New Madrid, Missouri, about 200 miles north of Memphis, Tennessee.

During a period of seven weeks in 1811–12, three earthquakes, each of estimated magnitude greater than 8 on the Richter scale, altered the course of the Mississippi River, and were felt in Chicago and Washington. Although no very large earthquakes have hit the American midwest since that time, one of the political aftershocks of the recent Loma Prieta earthquake in California (see *Nature* **341**, 676; 1989) has been an effusion of public concern over the dangers of earthquakes throughout the country. In order not to be accused of geographical chauvinism, Congress held a hearing last week to discuss the activity of the New Madrid fault zone, and the readiness of local authorities to cope with a catastrophe.

Unlike the geological instability of the west coast, which occurs at the boundary of different tectonic plates, faulting in the New Madrid region is caused by a mid-plate rift. As in the Mid-Atlantic and Red Sea rifts, subterranean material wells up as lateral forces pull the plate apart, but the New Madrid fault, according to Dallas Peck, director of the US Geological Survey, is now virtually static. The faults are buried beneath alluvial deposits carried down by the Mississippi, making the system complex and unpredictable.

Empirically, faults between magnitudes 6 and 7 are estimated to occur about every 90 years. As recent events in California and Armenia have demonstrated, the difference between disruption and disaster from earthquakes of this magnitude has much more to do with building codes and education than geology (see *Nature* **337**, 107; 1989). Arch Johnston, director of the Center for Earthquake Research and Information at Memphis State University, said at the hearing that progress on a local statute enforcing tougher building standards had accelerated remarkably after the Loma Prieta earthquake, and that new laws could be in place by the end of this year. But those measures apply only to the city of Memphis and its local county, and contain no requirements for retrofitting of existing buildings.

Although these issues and others, such as the availability and cost of earthquake insurance, were seriously discussed, it is unlikely that any real initiatives will emerge from last week's hearing. As Don Ritter (Republican, Pennsylvania) put it, "the 1812 New Madrid earthquake is probably as far from the thoughts of the citizens of Memphis, Tennessee, as the 1812 overture is from the citizens of Nashville".

David Lindley

LOMA PRIETA EARTHQUAKE

Stanford in squabble over relief funds

San Francisco

STILL reeling from the physical damage, estimated at $160 million, suffered in the Loma Prieta earthquake on 17 October, officials at Stanford University, 40 miles south of San Francisco, found themselves on the public relations defensive last week after a senator in the California legislature maligned them for a lobbying campaign to get state relief funds that, it was suggested, were better deserved by more needy groups.

The controversy started when Democratic state senator Bill Lockyer tried to limit to $1 million the amount of state aid a private, non-profit institution such as Stanford could receive. But he quickly ran into opposition as the package wound its way through the layers of state government. First, the limit on funds was raised to $5 million. Then, when the bill was finally signed into law earlier this month by Governor George Deukmejian (Republican), the limit was waived altogether unless relief funds ran out.

The final form of the law upset Lockyer, who directed his anger at Stanford University. "Stanford was the main lobbying force arguing against any cap at all", he claimed, adding that representatives of the university attended all the discussions: they were "in the common hearings, at the lobbying gate, visiting people's offices and buttonholing them for the whole week". The senator was incensed that the wealthy institution used its considerable legislative weight to try and push through its package, especially as it has a $1,800-million endowment. "I'm not a Stanford basher", Lockyer said, "I think it's a wonderful institution, and their contributions to our culture are enormous." What irked him, he said, was that "a lot of other people or organizations that are less fortunate don't have similar capacities to take care of their own problems". For their part, Stanford officials were stunned by the accusations, and have written to the editors of both the campus paper and a local newspaper defending their actions. "I'm really surprised that this has somehow become newsworthy", said spokesman Larry Horton. He acknowledged that the cap was the subject of a major debate in the Senate, but said that the university was only supporting what it thought was the most reasonable position.

Robert Buderi

MAX-PLANCK SOCIETY

Zacher is new president

Munich

THE senate of the Max-Planck Society (MPS) last week selected Hans Zacher, now director of the Max-Planck Institute for foreign and international social law, as the organization's new president. Zacher, who was the only nominee presented to the senate, will begin a six-year term in mid-1990. He is the first president to emerge from the humanities section of the MPS in its 41 years, and succeeds Heinz Staab, who returns after his six-year term to the Max-Planck Institute for Medical Research in Heidelberg.

MPG/Filser

Zacher will take office in mid-1990.

With 62 institutes and an annual budget of DM1,240 million ($670 million), MPS is the largest research organization outside the universities in West Germany. Only ten of the institutes are in the humanities, the rest covering biology and medicine, chemistry, physics and technology. Zacher, who is 61 and a pioneer of social law research for whom the institute for foreign and international social law was founded in 1980, now faces problems familiar to the heads of all science organizations in West Germany: an expected wave of retirements among directors late in the next decade, accompanied by the need to find positions for a flood of young researchers. Some MPS members worry that the organization of the society is not flexible enough to handle all the changes (see Nature 340, 335; 1989).

Steven Dickman

PERESTROIKA

Market economy needed

Moscow

A transition to a regulated market economy, along with a large-scale decentralization of management, is the prescription given by the Soviet Academy of Sciences for the ailing Soviet economy.

These changes, and a number of urgently needed steps to bring them about, were recommended after a round-table discussion held at the academy on 9 November, and attended by economists, People's Deputies (members of parliament) and officials from federal ministries and departments.

Many of the recommendations are aimed at 'westernizing' the consumer market: to bring money that is at present held as individual savings back into circulation, housing and land should be put up for sale, and businesses should offer shares to the public; more industrial manpower and resources should be allocated to the production of consumer goods and services; more consumer goods should be imported directly, instead of being made from imported raw materials. At the same time, the academy recommends, measures should be taken to restrain wage growth and restrict credit. With these changes, experts say, inflation will fall from its present rate of 8–10 per cent per year to 5–6 per cent, and consumer shortages will largely disappear in three years.

To support this new strategy of gearing the Soviet economy to the needs of the population, there must be stable investment in heavy industry, a reduction in the output of mines and a slashing of military spending: this will permit the present investment in consumer goods production and the service sector to be doubled, and still allow a 50 per cent increase in social spending.

But the authors of the academy report, which is to be submitted to the Supreme Soviet and to the government, say that it will be impossible to maintain a Western-style consumer goods market without complete reform of the pricing system and the creation of free markets in investment and credit, in manpower and in convertible currency. The present system of price-setting, in which the prices of fuels, raw materials and chemicals, for example, can be greater or less than the going world rate by as much as a factor of two, is fundamentally flawed, the report declares, and should be gradually changed. By 1995, the ratio between the numbers of products with fixed and market prices could be 30 to 70.

With price reform and a massive changeover to wholesale trade, the academy believes that the range of products in short supply would begin to shrink and most goods would then begin to circulate freely on the consumer market. But some economists are more cautious, believing that a radically revised system can succeed only if shortages, and the consequent social tensions, are alleviated first. That problem is inseparably linked with the question of state subsidies for foodstuffs, which now total over 100,000 million rubles a year. Retail price reform would allow this figure to be reduced by as much as 75 per cent, but the population would have to be protected from the inevitable increases in foodstuff prices by comparable increases in income.

Concomitant with all this, a commercial banking system would have to be installed. The report foresees that a free money and investment market, coupled to a realistic foreign exchange rate, could take shape between 1993 and 1995.

But a genuine market economy has its own set of ailments, notably inflation, unemployment and bankruptcy. To safeguard the population against these negative effects of reform, the academy proposes, for example, linking wages wit some kind of regularly revised an published consumer price index. Th future of *perestroika* depends, the economists assert, on the ability of th government to overcome the public's distrust of the state, which means that th state must give up its universal proprietorial rights and concede the idea o diversified ownership.

The report criticizes draft Soviet legislation on leases, arguing that it constitute an insufficiently radical reform of stat property, and that it should be modified t allow unreservedly the rights of leaseholders to control income from a lease acquire productive assets and hire labour provided only that terms are agreed i advance. The academy also comments o a proposed new tax system, and says tha the minimum taxable income should b higher and the maximum tax rate, now 8 per cent, lower. **Oleg Borisov,** Novost

NUCLEAR PROLIFERATION

Nuclear technology for Brazil

Munich

Despite opposition charges that Brazil has used West German nuclear technology to develop bomb-making capabilities, West Germany last week extended, for five years, an agreement to provide Brazil with more technology. The government withstood a fiery attack in the Bundestag (parliament) from the opposition Social Democrats (SPD) and Green Party, who claimed that the government was pandering to industry. Brazil is a significant importer of West German nuclear technology, which has lost its domestic market.

The government says there is no proof that Brazil can make nuclear weapons, and points to a clause in the Brazilian constitution that forbids Brazil from developing nuclear weapons.

Ulrich Irmer (Free Democrat) cited in an October debate in the Bundestag a report prepared by the West German intelligence service *Bundesnachrichtendienst*, according to which Brazil is not capable of producing weapons-grade uranium.

Nuclear reactors in Brazil are subject to oversight by the International Atomic Energy Agency in Vienna, and the West German government says it is satisfied with these various safeguards. But the Social Democrats (SPD), who have been pushing for tighter export restrictions in sensitive areas such as nuclear power and chemical weapons (see *Nature* **337**, 678; 1989), say that government is not telling the whole story. Because Brazil has not signed the Nuclear Non-Proliferation Treaty (NPT), SPD Bundestag member Edelgard Bulmahn argues that it ma have a military programme intent o developing weapons. Only by signing the NPT, which would allow internationa monitoring of all its nuclear facilities could Brazil give a clear signal that it doe not intend to develop weapons.

The SPD cites the Brazilian military' so-called 'parallel programme' t develop nuclear technology as a threat and says that a reorganization of Brazil' nuclear programme in 1988 legitimize the drive toward nuclear weapons. Bu Claus Jäger (Christian Democrat), wh accused the opposition of trying to "stir u a scandal", says that government is "convinced" that nothing significant ha changed. The original West Germa agreement, signed in 1975, was to provide up to eight nuclear reactors as well a enrichment and reprocessing technolog to Brazil over a 15-year period.

Financial problems and technical hitches have led to a scaling-down, and Brazil now plans to build just three reactors.

Nevertheless, Brazil imports a considerable amount of nuclear technology from West German companies. Sources in the Bundestag estimated the value at DM 600 million ($330 million) in 1989 alone, and the collapse of the market for nuclear power plants in West Germany, along with the decision not to reprocess spe nuclear fuel at home, makes the nuclea industry more dependent than ever on foreign markets. **Steven Dickman**

■ For more on Brazil's nuclear capabilities, see page 374 of the supplement "Science in Brazil".

FRAUD IN SCIENCE

Indian zoologist suspected

New Delhi

A researcher at Bharatiyar University in the south of India is being investigated by the university for a deception allegedly practised on his British and US colleagues during scientific visits last summer. Professor G. Sundara Rajulu, head of the zoology department at Bharatiyar, has been accused by two US scientists of having 'perpetrated a hoax' by pretending that blood samples he collected in Britain came from a rare worm he claimed to have found in India.

The allegations were made in a letter written on 10 October to the chancellor of Bharatiyar University by Joseph Bonaventura, director of the marine biomedical centre at Duke University, North Carolina, and Charlotte Mangum, professor of biology at the College of William and Mary in Williamsburg, Virginia. Rajulu had spent two weeks as a guest in their laboratories during May and June.

The affair began when Rajulu contacted Bonaventura and Mangum, claiming that he had found haemocyanin, the copper-containing analogue of haemoglobin, in the rare Himalayan species *Eoperipatus Weldoni* a member of the phylum Onycophora. He wanted to bring some samples to the United States for further analysis. Onycophora are thought to be a link between annelid (legless) worms and arthropods, and the presence of haemocyanin could shed light on the link. Bonaventura and Mangum invited Rajulu to bring some live specimens to the United States for further analysis.

But when he reached their laboratories, Rajulu had no live specimens, and said they had been confiscated by US customs officials. Instead he produced a frozen blood sample and a preserved specimen of what he claimed was *E. Weldoni*.

The blood sample did indeed contain a haemocyanin typical of crustaceans, but on further examination turned out to have a general composition more characteristic of marine than land organisms. Mangum then discovered that Rajulu had come to the United States after a visit to Muriel Walker at the University of Leicester in England. An exchange of letters with Walker revealed not only that the blood sample Rajulu took to the United States was from a British shore crab, but also that he had told his UK hosts the same story about confiscated specimens that he had related to Bonaventura and Mangum.

Rajulu was supposed to have brought to Walker and her colleague Robert Harris live specimens of some other Himalayan creatures, but said that UK customs officials had confiscated all but one partial sample, which Harris later ascertained to be part of a common marine worm. The blood sample that Rajulu presented to Bonaventura and Mangum was collected at his request by Harris.

Rajulu admits he has been served with an "explanation notice" by Bharatiyar University, but is surprised at the fraud allegation because, he says, Bonaventura and Mangum expressed no doubts about his discovery of haemocyanin during his visit to their laboratories. But on 26 July, after he had gone back to India, Rajulu sent Bonaventura a brief note saying that "in my anxiety to do things I committed a lot of mistakes" and apologizing for the matter. But no other explanation of the incidents was offered.

The Indian National Science Academy has also been sent a copy of the allegations but has yet to take any action. Bonaventura and Mangum agree that no great damage was done by the incident, apart from the waste of several researchers' time, but want to have the matter investigated because Rajulu is in the habit of arranging annual scientific visits outside India. Mangum says she knows of trips to Korea, Belgium and Vancouver, but has had no success in discovering if any similar deceptions occurred on those trips.

K.S. Jayaraman

JAPAN

Huge profit from drugs

Tokyo

JAPAN's hospitals and doctors are reaping enormous profits through sales of prescription drugs because pharmaceutical companies sell the drugs to medical professionals at a price far below the government's official reimbursement prices, an official of the Ministry of Health and Welfare admitted in the Diet earlier this month. The discounting practice has long been known to exist and is a major driving force behind excessive consumption of prescription drugs in Japan. But it is the first time a government official has put a figure on drug profiteering.

During a session of the auditing committee of the House of Representatives on 8 November, Tatsuhiko Sakamoto, director-general of the Health Insurance Bureau of the Ministry of Health and Welfare, under questioning by a member of the opposition Komeito party, revealed that the profits amount to about ¥1.3 million million (nearly $10,000 million) per year. Medical institutions charged patients and the health insurance system about ¥5.15 million million yen for drugs in 1987 on basis of the ministry's official prices, but Sakamoto estimates the actual cost to the institutions to be only ¥3.83 million million.

Unlike medical practice in most developed nations where doctors are not allowed to sell drugs, nearly all hospitals and general practitioners in Japan have their own in-house pharmacies. To promote sales, pharmaceutical companies sell drugs to medical institutions at below the government's official price (the price that determines charges). There is thus a strong incentive for doctors to overprescribe and to prescribe drugs unnecessarily because they make a profit on every sale.

The profiteering and overprescription is a massive drain on the national health system and also has serious consequences for the health of the nation.

One example is antibiotics. In the West, doctors try to avoid prescription of antibiotics because of the risk of development of antibiotic-resistant bacteria. But in Japan, antibiotics are liberally prescribed for all sorts of minor infections.

Another example is blood products. In the early 1980s, consumption in Japan surged with the import from the United States of cheap blood products which companies then sold to medical institutions at well below the government's official price. Among them were blood coagulants infected with AIDS, and most of Japan's 1,000 or so AIDS patients and carriers of the virus are haemophiliacs infected by the imported products.

For years, the ministry has been trying to separate the dispensing of drugs from doctors (a process called *bungyo* in Japanese). One approach has been to encourage the use of so-called 'legal prescriptions' which are handled by pharmacies outside the hospital. In response, large numbers of 'second pharmacies' have sprung up — although at first sight independent, they are in fact linked to medical practitioners. The doctor's wife, for example, may own the pharmacy.

Doctors and hospitals maintain that profits from drug sales are essential for management of their institutions. But slowly they are being brought 'reluctantly' towards full-scale *bungyo*, says Luke Gander of Pharma Japan, a newsletter for the pharmaceutical industry. But Gandor does not expect complete separation of doctors and pharmacies for 10 to 15 years.

There are too many vested interests to bring about rapid change. Pharmaceutical companies, both foreign and Japanese, are happy with their high sales in Japan. Doctors are happy with their profits. And the unquestioning Japanese public are happy to take home their bagfuls of unlabelled drugs after they visit the doctor. As the representative of one British pharmaceutical company observed, "It doesn't seem to do them any harm. The Japanese have the highest life expectancy in the world."

David Swinbanks

GENETIC TESTING

CF screening premature?

Washington

At its annual meeting last week in Baltimore, Maryland, the American Society for Human Genetics (ASHG) issued a statement urging caution in the use of screening tests to alert couples to the presence of a genetic defect associated with cystic fibrosis (CF) and the consequent possibility that their children might be born with the disease. Geneticists agree that testing should be offered when either partner has a close relative affected with CF, but say they have "serious reservations" about widespread screening.

The race to develop a new test was prompted by the announcement in August of the cloning of the gene for CF by researchers at the University of Michigan, the University of Toronto and the Hospital for Sick Children, Toronto (see *Science* **245**, 1059; 1989). Before then, CF could be detected only using conventional linkage analysis, which was available only to those with a family history of CF and with an affected relative still living. The discovery of the genetic mutation meant that a quick and cheap test could be developed based on a DNA probe specific for the mutation.

But the new test can still detect only about 70 per cent of CF carriers, because only that proportion of victims of CF have the identified mutation. Some researchers say that there should be no widespread screening for CF until the identification of additional mutations, which is expected within a year; others argue that the test is valuable despite its shortcomings and that widespread screening is worthwhile.

At its meeting last week, the ASHG erred on the side of caution, saying general screening should wait until a larger proportion of CF carriers can be detected and also until there is a better idea of the education and counselling that could be given to CF carriers. To this end, says the ASHG, pilot screening programmes should be started as soon as possible. Peter Lanciano, the general manager of Integrated Genetics Inc., one of the companies providing a CF test, endorsed the ASHG statement, saying that it would do a disservice to the public to rush ahead with widespread screening "just because you can do part of the work".

He said that the company will refuse to provide the test to anyone who does not have a family history of CF. Frank Fujimura, scientific director of molecular biology at the Nichols Institute said that the institute would advise against taking the test but would not refuse it. But he admitted that resources are not available for providing counselling services on the scale required for routine widespread carrier screening for CF.

Fujimara said that although the laboratories involved are highly regarded, there is widespread concern at the lack of mechanisms to ensure that standards are maintained. In last week's statement, the ASHG also called for centralized quality control; both it and the College of American Pathologists are considering some kind of certification procedure for laboratories. The ASHG is now establishing a working group to study all the issues raised by the new test and produce a report next year. **Christine McGourty**

HUMAN FRONTIER PROGRAM

Strasbourg office opens

Tokyo

The long-awaited opening of the office for Japan's Human Frontier Science Program in Strasbourg, France was due to take place on Tuesday, 21 November (see *Nature* 340, 329; 1989). As of 17 November, more than 130 research groups led by scientists from the seven summit nations (Japan, United States, Canada, Britain, West Germany, France and Italy) and the European Communities had applied for the 20 or so $500,000 grants that will be available, for research on the brain and molecular recognition, in the first year of full operation of the programme. Because of delays in opening the office in Strasbourg, the deadline for grant, fellowship and workshop applications will be extended to the end of this month, according to Toichi Sakata, director of the Frontier office at the Science and Technology Agency. David Swinbanks

BIOTECHNOLOGY

Celltech to change hands?

London

The major shareholder in Celltech, the largest UK biotechnology company, is to sell its 36.4 per cent holding. It is considered likely that potential buyers will bid for all of Celltech's shares.

British and Commonwealth Holdings has been a shareholder from the start of Celltech in 1980. It has decided to sell its shares for "strategic reasons", and has asked the board of Celltech to identify possible buyers.

The news follows an announcement by chief executive Gerard Fairtlough, that he is to stand down from the company he has led from the start. Celltech, best known for its monoclonal antibody products, recorded its first profitable year in 1987. Last year was also marginally profitable.

Peter Newark

FOOD IRRADIATION

Limited use approved in India

Bangalore

India's National Monitoring Agency (NMA), set up by the government to study the problems associated with food irradiation, has approved limited irradiation tests with a view to general commercial use of the process, which kills bacteria that cause deterioration of food. Food irradiation became controversial in India following public protests over the import by India of Irish butter-oil that was alleged to have been contaminated by fallout from the Chernobyl nuclear explosion.

But now M.R. Srinivasan, chairman of India's Atomic Energy Commission, says that the NMA has approved the irradiation of spices and frozen sea food for export, as well as the irradiation of onions for the home market. To further the prospects of commercialization, the Department of Atomic Energy has joined with Gujarat Agro Industries Corporation and the Spices Board of Kerala, and intends soon to set up gamma irradiation facilities in a number of places in India.

Radhakrishna Rao

SOUTH AFRICA

New CSIR president

Cape Town

Dr Brian Clark, 41, has been appointed president-elect of the South African Council for Scientific and Industrial Research (CSIR). The appointment was announced earlier this month by the Minister of Trade, Industry and Tourism, Mr Kent Durr. Clark will succeed Dr Chris Carberts, the current CSIR president, in September next year. He will be the sixth and youngest president of the organization, which is the largest of South Africa's three major research councils.

Clark joined the council's National Physical Research Laboratory as a technician after leaving school in 1965, and completed both his undergraduate and postgraduate training at the University of Pretoria as a part-time student.

Clark's present post in the CSIR is executive head of research, development and implementation (RDI). In this capacity he has been responsible for leading last year's restructuring of the organization into 12 technologically orientated divisions in the hope of attracting more research contracts from the private sector and government departments (See *Nature* 332, 197; 1988). In the current financial year, he expects outside contracts to amount to R174 million, accounting for 35 per cent of the RDI's budget.

Clark said in a statement that he hoped "to manage the CSIR in such a way as to make a major contribution to the ongoing development of South Africa".

Michael Cherry

WEST GERMAN UNIVERSITIES

Battle for youth plan

Munich

AT the same time as West German education minister Jürgen Möllemann has become the favourite of science organizations, he has become the bane of the education ministers of the *Länder* (states) in a simmering territorial dispute. Now he faces an all-out battle to gain support for a programme to support young researchers that could shape research and teaching at West German universities for the next 30 years.

Education had traditionally been a rump ministry when Chancellor Helmut Kohl chose Möllemann (Free Democrat or FDP) for the post in March 1987. When the ministry was created in 1972, the weekly newspaper *Die Zeit* wrote that if West Germany needed separate ministries for research and education, it should also set up separate ministries for letter post and parcel post. But circumstances besides his own ambitious nature helped Möllemann to overcome these hindrances: the FDP saw in the post a chance to put its liberal programmes across, and the federal budget was beginning to fatten after several lean years.

Möllemann soon realized that there is little for an ambitious education minister to do. His official brief covers just a few areas, including funding of the Deutsche Forschungsgemeinschaft (DFG), and construction at universities. So he began to try to steal areas of authority from other jurisdictions, mainly the *Länder* and, to a lesser extent, the Research Ministry.

Möllemann's style is to find a topical issue, overcrowding at universities for example, which brought students onto the streets a year ago in larger numbers than had been seen in 20 years (see *Nature* **336**, 704; 1988), then identify himself with the issue in the press — in this case by visiting the Munich university at the height of the unrest, where he was pelted with tomatoes. Finally, he makes a proposal to solve the problem: combined federal and *Länder* investment to provide more teachers and facilities in overcrowded fields such as business studies.

Möllemann's *Staatssekretär* Fritz Schaumann lists a number of achievements of which the minister is most proud: an increase in federal aid for students from middle-income families; the establishment of 40–60 graduate colleges to encourage more rapid progression to the PhD level; support for more research at technical colleges; and big increases in university building funds and for DFG.

For the long-term health of university research and teaching, the most important challenge is the plan to support young researchers for up to ten years until large numbers of professorships become available (see *Nature* **341**, 94; 1989). This could help to persuade the best young people to remain at universities instead of going into industry or business. Despite criticism from finance ministers and even some education ministers in the *Länder*, the plan still has a fair chance of being passed at a 21 December 'education summit' with Kohl and *Länder* politicians in Bonn.

But the government will be hard pressed to find the DM6,000 million ($3,200 million) that Möllemann wants for the plan. Priorities in Bonn have shifted since the huge influx of East German refugees began this summer.

West German education minister, Jürgen Möllemann.

Another important task, according to Schaumann, will be smoothing the way for the integration of West German courses of study into the European Communities, a tricky problem complicated by the differences in norms among the *Länder*, which fear losing autonomy to Brussels as well as to Bonn. Möllemann's activities have disturbed the *Länder*, to whom education policy is one of the last remaining bastions of power in an increasingly centralized country.

"Compared to what he has promised, the results are meagre", complains Thomas Goppel of the Christian Social Union (*Staatssekretär*) in the Bavarian Ministry of Science. "If he wants his programmes to succeed, he should discuss them with us first," says Goppel, "he achieves ten per cent of what he has promised and then sells it as success." And "Möllemann is only interested in headlines", says Uwe Knüpfer of the Social-Democrat-run science ministry in North Rhine-Westphalia: "he ignores issues where behind-the-scenes negotiations would be more helpful than publicity campaigns." Goppel and Knüpfer say that Möllemann has done more for his own career than for students and professors.

But Möllemann has admirers in the science organizations. DFG president Hubert Markl says the minister "should be given credit" for this year's budget increase; Markl's pleading fell on deaf ears in the parliament and the government until Möllemann made DFG an issue. And Dieter Simon, chairman of the *Wissenschaftsrat*, says Möllemann's concern for science has done a lot of good: "even though his suggestions are not always the best, the fact that he keeps making them is a big help." Even critics such as Goppel agree that Möllemann has given a higher education a "new valuation" in the public mind. But both supporters and critics say that the biggest test for Möllemann — gaining federal and *Länder* support for his ideas — lies ahead. He cannot afford to walk away empty-handed from the 21 December education summit or the lasting effect of his contribution will, in the words of Knüpfer, "vanish in a puff of smoke". **Steven Dickman**

RESEARCH FUNDS

More money for UK science

London

As part of UK Government expenditure plans announced last week, about £60 million will be added to the science budget for each of the next three years. Of the £61 million added to next years budget, £17 million is earmarked for the construction of a new research vessel for the British Antartic Survey. Small sums are also allocated to the British Geological Survey, to enable it to set up a National Geological Information Service.

Next year's budget will now be £897 million, 27 per cent more than was spent in 1988–89. The Advisory Board for the Research Councils, which had been pushing for an even greater increase, will be deciding on how the extra money should be distributed among the research councils.

A simultaneous announcement of extra expenditure on higher education anticipates a predicted increase in the number of extra students. A system of student loans is to be introduced as part of the plan to boost numbers. Peter Newark

Reasonable doubt?

SIR—George F. Wood's letter (*Nature* **341**, 100; 1989) suggests that some of your readers may not be aware of the legal profession's understanding of certain "issues involving probability". A substantial body of case law and commentary addresses the two issues he identifies. On the question of "what probability represents the 'reasonable doubt' beloved of the legal profession", see, for example, *Branion* v. *Gramly*, 855 F.2d 1257, 1263 n.5 (7th Cir. 1988) ("reasonable doubt means 0.9 or so, with adjustments depending on the gravity of the offense"); J. Kaplan *Stanford L. Rev.* **20**, 1065–1092 (1968). On the "purely statistical" proof of an elevation in "death rates due to . . . external circumstances", see, for example, *Sindell* v. *Abbott Laboratories*, 26 Cal. 3d 588, 163 *Cal. Rptr* 132, 607 P. 2d 924 (1980); D. Kaye *Am. Bar Fdn Res. J.* 487–516 (1982).

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No paying twice

SIR—Henry Gee's News item (*Nature* **342**, 110; 1989) correctly explains the background and purpose of the programme of the European Science Foundation (ESF) on the molecular neurobiology of mental illness. ESF is asking for FF10 million ($1.6 million) from its member organizations to run this programme for five years. Clearly this sum will not pay for all of the science involved; in each ESF country, the cooperating scientists will have to use normal national routes to raise the research funds required.

The ESF's FF10 million will be spent largely on coordination: interchange between scientists, collection of new pedigrees, computer services and the initiation of Southern blot analysis of chromosomal regions outside the normal interests of mental illness specialists, to ensure complete mapping of the genome.

There can of course be no question of anyone "paying twice" for the same work – no ESF member organization would do that.

Gee reports the concern of scientists about two points. First, I can reassure colleagues that the formal code of practice of the programme contains provisions for allotting particular regions of the genome to research groups that can justify a deep involvement with that region. Otherwise allotment will be 'random' to ensure both fairness and complete coverage of the genome. Second, the data facility of the programme will not perform any linkage analysis unless help is specifically requested by the members supplying the data.

We hope that this programme will help to identify innate risk factors in mental disease and provide a tool for the refinement of psychiatric diagnosis. These are high ambitions; to further them, we must of course satisfy both our professional colleagues and the proper concerns of funding organizations. We believe we are doing so.

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Purge the bombers

SIR—The problem of terrorist bombings of airplanes in flight might be solved by a simpler and possibly more effective means than large, expensive thermal neutron analysers (TNAs). Specifically, the solution can make use of the elementary principle in chemistry that oxidation, including spontaneous combustion of plastic explosives, requires oxygen. Exclusion of oxygen, rather than or in addition to bombs, from airplane cargo holds could be accompanied by purging with nitrogen, an inert, completely non-toxic and non-polluting gas comprising nearly 80 per cent of the Earth's atmosphere. Perhaps more realistically, the large bins used to contain checked passenger luggage could be purged, and the purge maintained through slow release of nitrogen from a liquid or (small and safe) compressed source within each bin.

This proposal to exclude oxygen from luggage bins can be economically implemented in airports of even the most technologically non-advanced nations. The universal problem of flight delays would be turned into a benefit by providing time before airplane take-off for nitrogen substantially to replace oxygen within each piece of luggage. To function, a terrorist bomb would have to carry its own oxygen supply, or use air contained within hermetically sealed luggage to support an exclusion of sufficient intensity to breach the nitrogen-purged luggage bin, whereupon the explosion could accelerate by using air in the cargo hold, assuming it has not also been nitrogen-purged. It should be easy to scan luggage for hermetic sealing and/or the presence of compressed oxygen. Nonetheless, it would be desirable to identify any or all conditions under which single pieces of luggage could carry sufficient non-compressed oxygen to sustain explosions of the intensity required to breach nitrogen-purged luggage bins.

In airports equipped with TNAs, nitrogen-purging can address the problem of unacceptably frequent false-positive TNA scans. Instead of manually searching numerous items of luggage yielding positive TNA scans, such luggage could be placed in nitrogen-purged bins, with some obvious trade-offs. As a final point, the feasibility of this low-technology solution can be tested in the short term, before major resources are committed to developing costlier high-technology solutions to be available only in the more distant future.

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Nobel infallibility

SIR—The debate over the fairness or otherwise of the award of this year's Nobel Prize for Physiology or Medicine is reminiscent of the controversy over the 1952 prize for the discovery of streptomycin. This was awarded to Selman Waksman alone, specifically for his discovery of the antitubercular antibiotic streptomycin. Streptomycin was in fact discovered by one of Waksman's research students, Albert Schatz. Schatz had senior authorship on the main papers that announced the discovery and also appeared on the streptomycin patents. In addition, Waksman and others signed sworn affidavits to the effect that Schatz was co-discoverer. Finally, following a lawsuit, Schatz was legally defined as streptomycin's co-discoverer.

When Schatz's colleagues learned that Waksman alone was to be awarded the Nobel Prize for streptomycin, they began lobbying to correct the injustice. Some leading scientists, including William Feldman, sympathized with Schatz, but felt it politic not to intervene on his behalf. Amazingly, in reply to probing on Schatz's behalf, the committee that had awarded the prize admitted that it had never heard of Schatz. So much for the investigative abilities of the Nobel prize committees. It seems that nothing much has changed over the past 30 years or so. Indeed, the rules of the prize mean that no corrections can be made; essentially the Nobel committees regard themselves as infallible, and do not admit to mistakes. Stehelin should be warned that the scientific establishment does not take kindly to the Nobel prize boat being rocked. The career of Albert Schatz was blighted by his attempts to gain due recognition for his work. We can only hope that the same fate does not befall Stehelin.

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Popper on Schrödinger

SIR—I have read, somewhat unwillingly, P.W. Atkins' review of a biography of my late friend Erwin Schrödinger, which appeared under the title "Physicist and philanderer" (*Nature* **341**, 193; 1989). I should be grateful if you would print this defence of a great man against the accusation of cowardice ("Schrödinger also appears to have been a coward . . .").

I do not know and I do not care who originated the accusation. But the accusation is absurd, as I will show. It illustrates how some people fail to understand the situation that prevailed in Germany and in Austria under Hitler.

Atkins writes of Schrödinger: "After fittingly succeeding Max Planck in Berlin in 1927, he (a Protestant) escaped to Oxford in 1933". This is a *suggestio falsi*: Schrödinger did not 'escape' from the Nazis, as did so many scientists (and others) who had been dismissed and who were threatened by a fate worse than dismissal. On the contrary, like Planck, von Laue and Heisenberg, Schrödinger was, in Hitlerian terminology, an 'Aryan', and like them he could have stayed in his position in Berlin and enjoyed wealth, power and influence. He did nothing of the sort. He gave up his position as the leading physicist in Berlin, and he chose, very soon after the dismissals had started in 1933, to join his 'non-Aryan' colleagues of his own free will. He did so with a public declaration expressing his disgust with Nazism.

Owing to the help of Professor Frederick Lindemann (later Lord Cherwell), he came to Oxford where I visited him in 1935 and 1936. I found him intensely unhappy. He was grateful to the powers that had made his reception at Oxford possible. But he was particularly unhappy about the scientific atmosphere there (or rather about the complete lack of it). He was not, of course, exclusively interested in physics: he was also very interested in classical scholarship. But he complained that, in Oxford, no serious discussion about scientific or scholarly problems was possible. It just was not 'done'. It was regarded as banausic. Instead, one gossiped about people — usually running them down — and often rejected them in spite of their obvious competence and devotion to their subject. Schrödinger rejected this kind of snobbery (as did Churchill); and some silly people interpreted his open rejection (the openness of an apparent refugee) as ingratitude towards Oxford, and even towards England, which Schrödinger loved. That he ever "abused" Lindemann, or that he showed him "ingratitude", I regard as absolutely impossible. But Schrödinger was unused to dissembling, was highly temperamental and even a bit irascible. And, as I know only too well from many discussions I had with him, he did not suffer fools gladly. But at the same time he was ready to make amends by showing special kindness. To say that he "considered himself to be God" is again absurd: he was very conscious of being far too frequently in the wrong, even morally.

During my visits to Oxford, when Schrödinger told me of his unhappiness, he also mentioned that he might leave Oxford and accept a chair in Austria which, in those days, was ruled by Schuschnigg who tried to keep the Nazis down. I implored Schrödinger to give up this mad idea. I told him that I was trying to leave Austria because Schuschnigg, who was after all a dictator like Mussolini and Hitler, was bound to succumb to Hitler. But Schrödinger thought that the allies would not let Hitler annex Austria — nor would Mussolini; and he said that, after all, if Hitler were to endanger Austria, he would emigrate again.

Not very long after this, I heard from Hans Thirring (my teacher in theoretical physics) that Schrödinger had declined Thirring's offer to vacate, in his favour, the chair in Vienna for a vacant chair in Graz, but that Schrödinger had himself accepted another chair in Graz.

In 1938, Hitler invaded Austria after a crisis of only a few days which left Schrödinger no time to escape (this time, the term 'escape' is appropriate). Being a known opponent of Nazism, he was at once in great danger. He was forced (like Galileo) to recant his anti-Nazi views; but being a little younger than Galileo was at the time, he managed an adventurous escape: he put a small suitcase with his most important documents into his old car and drove to the Italian frontier, where he left his car standing in a side road and crossed the frontier with his wife on foot. Once on the Italian side, he took a train to Rome. There he telephoned Dublin, trying to contact Eamonn De Valera, the Irish prime minister, who had invited him before. De Valera, he found out, was in Geneva. So he rang Geneva and, with some difficulty, reached him. He told him about his situation, and that his money was running out rapidly while he was telephoning. De Valera said he should at once take a train to Geneva. On the Italian–Swiss border, Schrödinger and his wife Annemarie were stopped by the *carabinieri*: they were forced to get out of the train, which left without them. They thought that the Nazis had alerted the Italian fascists. They were both searched, but they were allowed to continue by a later train. (It turned out that because they had hardly any money on them, they had been suspected of trying to export money illegally.) Once across the border they were safe.

Letters submitted for Correspondence should be typed, double-spaced, on one side of the paper only.

Some of my ('Aryan') friends in Austria managed to leave under tragic and dangerous circumstances. One of my closest friends, Otto Haas, a lieutenant in Hitler's army during the war, tried to organize a resistance movement and was hanged in 1944.

Some of my friends made their peace with Nazism. They were in danger for they had been *Judengenossen* (associates of Jews). Who is to blame them? Who is to call them cowards?

But Schrödinger was not one of them: he left Austria soon after its annexation under the most dangerous circumstances, even though he would have been welcomed by the Nazis had he recanted. Galileo did recant. Who dares to call him a coward?

A last remark on poetry, on *What is Life?*, and all that.

When in 1927 I first read Schrödinger's *Abhandlungen zur Wellenmechanik*, I was overwhelmed, not merely by the beauty of his theory, but by the sheer poetry of it: I knew then that he was a real poet. When, long after his death, I read some of his poems, I remembered, and felt confirmed in my opinion.

When I read, 40 years ago, *What is Life?*, I was greatly excited. But I soon realized that this was a kind of manifesto without much content; that Schrödinger the poet had run away with Schrödinger the scientist. After all, he was inspired by the views of Max Delbrück, and the book was meant as a popularization of Delbrück's ideas. (Schrödinger's own suggestion that what characterized a living organism was that it sucked up negentropy was not to be taken seriously, for every washing machine does that, without coming to life.) However, the book worked as a biologist's manifesto, and inspired many young people: no doubt, Schrödinger's poetic powers succeeded where, as I believe, his biology failed. Here, as everywhere else, I disagree with your reviewer. (But I largely agree with Linus Pauling, and more or less with Max Perutz, in their contributions to *Schrödinger*, edited by C. W. Kilmister, which was published by Cambridge University Press in 1987.)

A last word: gossip about Schrödinger's philandering is not my business; nor, I suggest, is it anybody else's business. And I find it hard to swallow that Annemarie Schrödinger is being made part of the gossip.

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The nature of science

Sir—While A. Travis (*Nature* **341**, 10; 1989) may be bemused by the Universe, he is certainly befuddled about the nature of science. It would seem that, to Travis, only when a model can be "tested experimentally can it be held as truth". I, and I am sure many other scientists, would take issue with this statement.

Science cannot and should not claim to be discovering truths. As fallible human beings and scientists, our best hope is to propose theories (or hypotheses) that are rigorous enough to withstand repeated testing and to be supported by the evidence that can be gathered. Many of the theories simply will not withstand the testing and will either be falsified outright or, more likely, be replaced by a new and better corroborated theory. This is the strength of the so-called 'scientific method' in attempting to understand the world around us. It cannot proclaim ultimate truths as Travis would suggest, for those who do so step out of the realm of science.

A more debatable issue is whether only experimental science is truly science. Both the aforementioned statement of Travis and a later comment that "untestable theories in science (such as the evolution of life through random diversification followed by natural selection) are permissible, but not if they involve forces from an unknowable source", suggest that Travis would hold to the stricter 'experimentalist' perception of science. By most scientists' perception of their profession, however, if a theory is untestable it is not science and is not permissible, at least within the realm of science. The statement only makes sense if one replaces "untestable" with "non-experimental". If this is done, I have less quibble with the statement, because much of testable science is in fact non-experimental. Every science, but some more than others, has an historical component that represents a unique series of events that took place in the near or very distant past. The experiment has been run, but the evidence is there and can be corroborated over and over again just as an experiment can hopefully be repeated.

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Sir—A. Travis feels compelled to invoke a 'creative force' when considering the mysteries of the observable Universe, in particular those aspects that appear least amenable to scientific investigation. I doubt, however, whether it is appropriate to label such areas of mystery as being particularly 'religious'. Such labelling is similar to that which became popular following the deistic movement of the early eighteenth century, in which 'god' or 'religion' were invoked to explain phenomena for which science had not yet found satisfactory theories. Not surprisingly, such a 'god-of-the-gaps' faded like the smile on the Cheshire cat as science continued its advance. The invoking of a 'creative force' to fill the present gaps in our scientific knowledge does not seem any more satisfactory. The task of science is to construct better and better 'maps' that will incorporate an increasingly sophisticated understanding of the world around us, and there is no reason to suppose that topics such as 'human intelligence' and the 'origin of life' will not be much better defined in future maps than they are on the rather poor ones that we have at present.

Those who laid the foundations of modern science in the seventeenth century did not look for gaps in their scientific knowledge in which to make room for their religious beliefs. Quite the reverse: they saw behind the complete 'map' of scientific knowledge a God whose continuing creative activity made it a worthwhile enterprise to look for consistency and reproducibility in physical phenomena in the first place. Their sense of awe at the world around them came not so much from its unexplained mysteries as from the fact that it was explicable, and could be expressed particularly in the language of mathematics. For example, Galileo saw God as the "divine mathematician", who had made a universe that was intelligible by the human mind, so that investigating "the true constitution of the universe" was "the most important and most admirable problem that there is"[1]. Three hundred years later, our investigations of the 'true constitution of the universe' have a very long way to go, but it is good to emphasize that religion, at least the kind of religion that Galileo espoused, still has no hidden investments in scientific ignorance. The role of religion and philosophy is to address the much broader questions of whether the very existence of the 'map' itself, with ourselves as part of it, has any particular meaning in a more ultimate sense, and also to tackle the vital ethical questions as to how our scientific knowledge should be used. Peter Medawar has recently reminded us that the scientific method itself is not equipped to provide answers for such questions[2]. But as we attempt to tackle them at other levels, the proposal of 'unknown, creative forces' as religious-sounding plugs for the gaps in our present scientific knowledge is as inappropriate for religion as it is for science.

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1. Drake, S (transl.) *Discoveries and Opinions of Galileo* 97, (Doubleday-Anchor, Garden City, New York, 1957).
2. Medawar, P. *The Limits of Science* (Oxford University Press, 1985).

Sahni visit denied

Sir—In normal circumstances, I would have ignored V. J. Gupta's statement (*Nature* **341**, 11–12; 1989) that my father, the late M. P. Sahni, had visited his (Gupta's) localities in 1964, as not being central to the main issues at stake in the matter of the 'peripatetic fossils'—see the article by Talent (*Nature* **338**, 613–615; 1989) and those by others (*Nature* **341**, 13–16; 1989). But the present situation is far from normal and warrants that the truth be made known regarding M. P. Sahni's involvement in the matter. Sahni had an unblemished and distinguished record of meticulously conducted research spanning nearly half a century. It is therefore all the more distressing that his name has been dragged into a controversy after his death, and this has provoked several of his friends, ex-colleagues and well-wishers to ask me to set the record straight.

The facts are that Sahni attended a seminar at Srinagar (Jammu and Kashmir) in October 1964. He left Srinagar on 16 October, seen off at the airport by Dr S.K. Shah, one of the organizers of that seminar and now professor of geology at the University of Jammu. During his brief halt, Sahni neither visited the graptolite localities nor did he accompany the post-seminar field excursion and I have documentary evidence to substantiate this.

The controversy over the 'peripatetic fossils' has caused intense introspection and soul-searching among Indian earth scientists in general and palaeontologists in particular. Open systems based on trust are fragile institutions prone to attack, but the strength of such systems derives from the internal reaction and response in the hour of crisis. By this yardstick, the national response has been spontaneous and extensive: exposure in the news media and the setting up of two major commissions of inquiry and other bodies of investigation. Introspection has led to action.

Ashoki Sahni
Centre of Advanced Studies in Geology,
Panjab University,
Chandigarh – 160014, India

■ In the article by U. K. Bassi (*Nature* **341**, 15–16; 1989) the author's institutional address was used in error. Although U.K. Bassi can be contacted at the Geological Survey of India, his personal address should have appeared in the article. □

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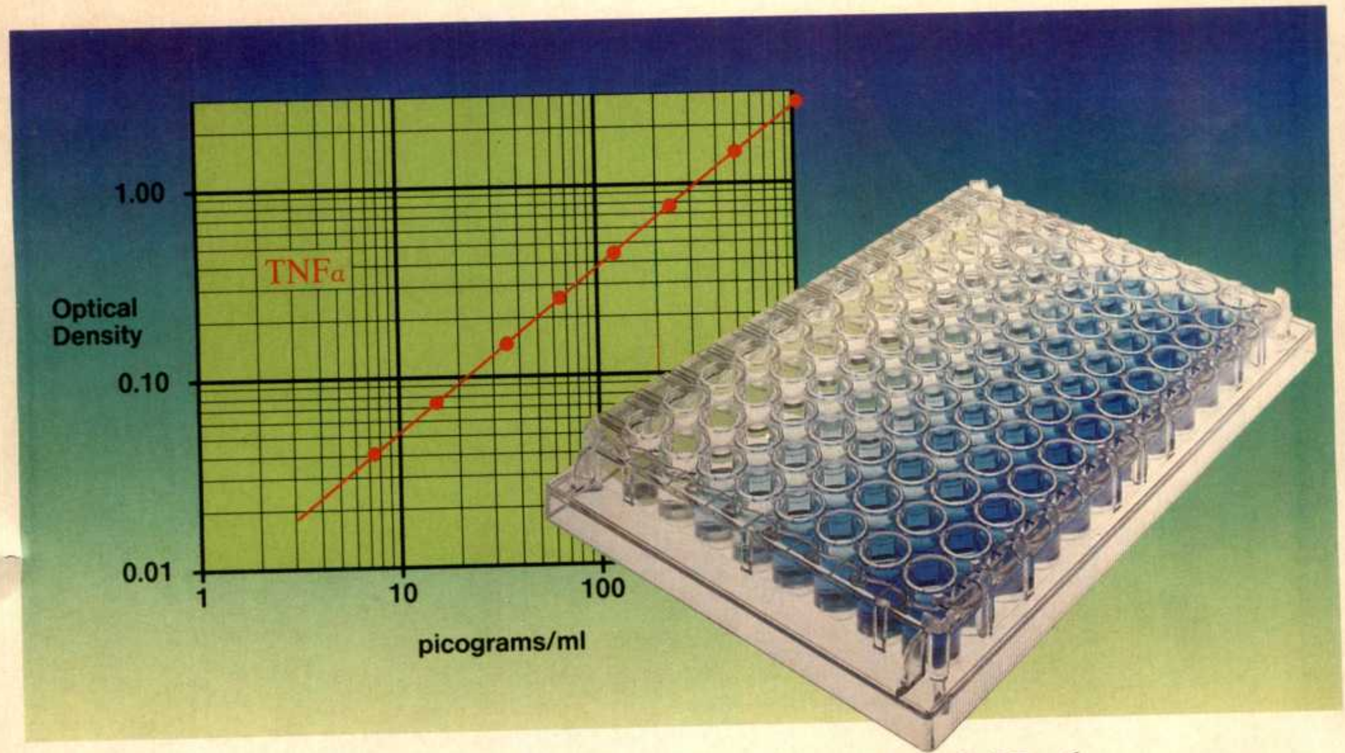

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Reader Service No.8

Disentangling the greenhouse

Continuing uncertainties about the working of the greenhouse enhancement give the lie to those demanding, at a meeting last week in the Netherlands, that the details of an international convention should be settled on the spot.

Melbourne

EVERYBODY seems to know that this is the part of the Earth's surface at which the Antarctic ozone hole reaches furthest north. While the observations of anomalous springtime ozone depletion outside the Antarctic Circle covers only a single season (this time last year), people seem convinced that this will be the beach summer of total block, of warmth without tan (see *Nature* **340**, 290–294; 1989). Australians do not lightly forgo such pleasures, which is why some wonder how long-lasting will be the resolve to keep ultraviolet away from skin.

But even in literate Australia, the confusion between the ozone hole and what is strictly the supposed enhancement of the normal greenhouse effect by atmospheric constituents opaque to infrared is as profound as elsewhere. There may be rough justice in that. The springtime Antarctic ozone hole, the reality of which seems well-attested by this season's observations, is at least a sign that a measurable property of the atmosphere is affected by the rate at which unwanted refrigerants are discharged to the atmosphere. If chlorofluorocarbons (CFCs) can affect the ozone layer, why should not the much more copious releases of other greenhouse gases, such as carbon dioxide, affect the climate?

The confusion is nevertheless a threat to good causes, some of which are also green. CFCs are greenhouse gases in their own right (and, molecule for molecule, are more effective than CO_2. But to a first approximation, ozone depletion, whatever its cause, does not affect the climate or even the weather. To a second approximation, of course, ozone depletion (and so CFCs) affect both climate and weather through the changed variation of temperature with altitude — the tropopause is lowered — but the consequences cannot easily be predicted quantitatively let alone disentangled from the data.

How can the distinction between the ozone hole and the enhancement of the greenhouse effect best be established as a part of general knowledge? Much might be done by the direct measurement of the spectrum of solar ultraviolet radiation reaching the surface of the Earth at different places and seasons. The most obvious practical impediments are those of calibration, which, in the absence of data from the past, must necessarily be absolute. Even so, it is remarkable that so little has so far been said about the flux of ultraviolet light reaching the surface of the Earth in even recent years. Paradoxically, if it were shown that ozone depletion means extra ultraviolet, it would be easier to separate that from the direct enhancement of the greenhouse.

While there should by now be no doubt that the next practical step should be the negotiation of an international convention to restrict the release of all greenhouse gases, the more obdurate impediments to understanding and prediction remain as obdurate as ever. It is maddening that the big uncertainties seem to change only slowly with the passage of time. Two stand out. The climate models can incorporate what is called average cloudiness, but the effect of real clouds, with real edges, on the heat balance in the troposphere could be qualitatively different. In principle if not in magnitude, the uncertainties are like those of estimating the role of clouds in nuclear winter (see *Nature* **318**; 99; 1985). That must remain a problem.

The other big uncertainty, at least so far as the greenhouse enhancement due to CO_2 is concerned, remains that of knowing the fate of whatever fraction of the atmospheric content is dissolved in the oceans every year. Increased conversion to inorganic carbon would be benign, solution as bicarbonate in a steadily deepening layer of warm water above the oceanic thermocline would be the opposite. That issue has been endlessly discussed, but there are few who at this stage would put their hands on their hearts and say they know the quantitative truth.

Even relatively operational questions, that of the movement of CO_2 within the oceans for example, are unresolved. That is nicely illustrated by an attempt last year, by Peter G. Brewer and Catherine Goyet of the Woods Hole Oceanographic Institution and David Dyrssen of the Chalmers Institute of Technology at Gothenburg, to measure the northwards flux of CO_2 in the North Atlantic (*Science* **246**, 477; 1989). The North Atlantic is usually supposed to be a substantial sink for the gas, which is supposed to be carried northwards by oceanic currents. But measurements across the Florida Gulf and at three stations along the 25th parallel in mid-Atlantic lead to the conclusion that while there is a substantial northerly transport, that is almost but not quite offset by transport in the opposite direction. The net northwards flux, at 260 million tonnes a year, is just over one per cent of the flux in either direction, or just under 5 per cent of the annual production of this greenhouse gas. This is much smaller than expected.

What can be the explanation? One is that the data may be insufficient. Three sets of measurements of CO_2 concentration with depth are not many, although the authors of the study have used previous survey data to interpolate between their stations. Another is that, while CO_2 will be released as the lower waters of the Gulf Stream reach the surface, it will be reabsorbed from the atmosphere as the Gulf Stream cools, reducing the partial pressure of the gas. Still another is that expectations are not yet fulfilled because there has not been enough time, since the onset of rapid greenhouse gas production half a century ago, for the deep waters of the North Atlantic to have travelled as far north.

In the circumstances, it must plainly be foolhardly to pretend just what the future course of the enhancement of the greenhouse effect will amount to. Better and more measurements will help, although none will be decisive. But if more were known of the variation of CO_2 concentration with latitude in the deep waters of the Atlantic, it might at least be possible to guess when transport into the North Atlantic atmosphere would become substantial. That would provide a means of telling just how quickly the international convention must be negotiated and brought into effect.

Questions such as these were plainly in the minds of some of those attending last week's meeting in the Netherlands, one of several meetings planned as preparation for the more formal diplomatic conferences to be held next year. Governments such as the British were pilloried, but unfairly, for having asked that the details of a convention — the date at which the further accumulation of greenhouse gases should be halted, for example — should await further study. That, of course, is entirely sensible. It is in nobody's interests that the economic disruption an international convention will certainly bring should be accelerated. It is not that there is nothing else to talk about, the demand on behalf of developing countries for fair shares in any quota system there may be for one thing.

John Maddox

IMMUNOLOGY

Tolerance: a second mechanism

Anthony L. DeFranco

A CENTRAL problem for the immune system is distinguishing foreign invaders from the body's self-components. Recognition of the former concentrates the destructive mechanisms of the immune system on the infectious organism; mistaken recognition of the latter results in autoimmune disease. On page 385 of this issue[1], Goodnow and co-workers report that immunological tolerance to self can be induced in mature B cells, thereby giving us good reason to think that tolerance involves at least two principal mechanisms.

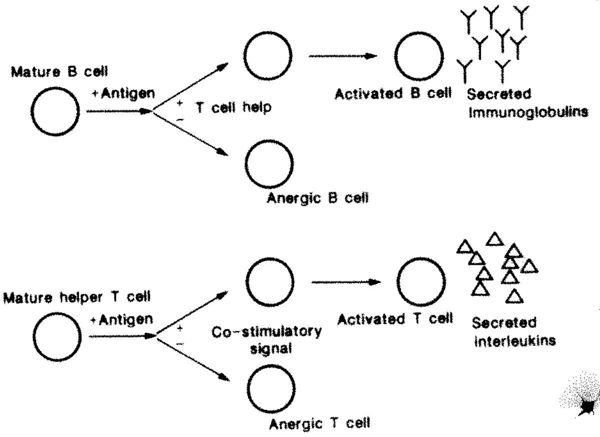
Discrimination between self and non-self can arise from a developmental switch in the response of lymphocytes to antigen: contact of antigen with the antigen receptor on immature B or T lymphocytes induces either death of the cell ('clonal deletion') or long-lasting unresponsiveness ('clonal anergy'). One problem with understanding how this mechanism could be fully responsible for immunological tolerance to self is that lymphocytes develop in limited anatomical locations (bone marrow for B cells and thymus for T cells), but once mature they circulate throughout the body. Thus, mature lymphocytes may encounter self-components that were not present at the sites of maturation, and that therefore could not have induced clonal deletion or anergy. An attractive solution to this puzzle is that contact with antigen may, under some circumstances, also induce unresponsiveness in mature lymphocytes, and Goodnow *et al.* provide perhaps the clearest demonstration to date of this form of tolerance induction.

We are currently in the third phase of the investigation of immunological tolerance. The first phase, ushered in by the studies of Owen *et al.*[2] with dizygotic twins of cows, established that tolerance is an acquired characteristic. The second phase involved analysis of tolerance at the cellular level. These experiments established that unresponsiveness was a property of the antigen-specific lymphocytes, that direct *in vitro* antigen exposure of B cells could induce unresponsiveness, and that clonal deletion or anergy was most easily achieved with immature B lymphocytes[3]. Less was known about tolerance of T cells, because it was harder to follow their activity. A great problem of the second phase was that antigen-specific lymphocytes could not be followed directly. In some experiments, antigen surrogates (such as anti-IgM antibodies) were used to circumvent this problem, but such an approach had inherent limitations.

With the advent of mice expressing transgene-encoded antigen receptors[4-7], and with the discovery that antibodies recognizing the products of certain gene segments ($V\beta$) can distinguish all T cells recognizing certain antigens[8], it is now possible to look at lymphocytes of a given specificity and analyse the effects when they encounter antigen. A great deal has already been learned from these third-generation studies. When immature T cells developing in the thymus encounter antigen and appropriate proteins encoded by the major histocompatibility complex (MHC), the result is clonal deletion[4,5,8]. Similarly, immature B cells expressing immunoglobulin transgenes are inactivated, either by clonal anergy[6] or by clonal deletion[7], if they develop in an animal expressing the antigen. These studies have shown the importance and efficiency of clonal deletion of developing T cells, and have put our knowledge of tolerance induction of immature B cells on a firm basis. In the 1960s, however, it was demonstrated that tolerance could be induced in mature animals, provided that antigen was introduced in the appropriate way (intravenously, for example)[9]. Moreover, it is hard to imagine how components that are apparently expressed only in selected tissues could be delivered to the sites of lymphocyte development in quantities large enough to induce clonal deletion or anergy. Thus, clonal deletion or anergy of immature lymphocytes is unlikely to be the only mechanism contributing to tolerance.

In the case of B cells, the problem of incomplete tolerance can, in principle, be sidestepped. Without the active participation of helper T cells, B cells do not make antibody responses to many antigens, including most self-components[10]. Antibody responses to many bacterial components do, however, occur without helper T cells being involved[10]. If there is a cross-reaction between such a bacterial antigen and a self-component that failed to cause efficient clonal deletion of those B cells recognizing it, then one might expect the production of anti-self antibodies. Indeed, this appears to occur in rheumatic fever, in which antibody produced in response to streptococcal infection cross-reacts with heart tissue, causing tissue destruction.

Goodnow *et al.* now demonstrate[1] that the immune system has a solution to this problem, at least in many cases. They took B cells expressing transgene-encoded membrane immunoglobulin specific for chicken lysozyme and transferred them from an environment lacking lysozyme into a host that expressed lysozyme as a self-component by virtue of a lysozyme transgene. When this was done, mature B cells encountered antigen without antigen-specific helper T cells. The result was that the B cells entered a state of profound unresponsiveness. Moreover, the B cells underwent a phenotypic change characterized by greatly decreased expression of one form of membrane immunoglobulin, mIgM, but continued expression of another form, mIgD. Goodnow and co-workers had previously shown[6] that if the immunoglobulin transgenic B cells developed in a lysozyme-expressing mouse, then the B cells underwent clonal anergy, and showed the same phenotypic change described above. Surprisingly, there was no apparent difference between encountering antigen as immature B cells and encountering it as mature B cells. From previous work, one would have expected a considerable difference in the amount of antigen required to induce clonal anergy, with less needed for immature cells. If B cells with low amounts of mIgM and high amounts of mIgD have all undergone clonal anergy, either as immature B cells or as mature B cells, then this is a common fate, as Goodnow *et al.* report[1] that a



A second mechanism for immunological tolerance. Many (but not all) lymphocytes that recognize self-components are inactivated if, at an immature stage, they come into contact with antigen. Lymphocytes that mature without this happening may come into contact with self or foreign molecules through their antigen receptors, which leads to cellular proliferation and immune responses (antibody secretion, interleukin secretion and so on) provided appropriate second signals are also received. For B cells, such second signals can come from helper T cells (in the form of interleukins) or, in the case of T-cell independent antigens, probably from macrophages. For helper T cells, the antigen-presenting cell (typically a macrophage, B cell or dendritic cell) provides a 'co-stimulatory signal' which is as yet unknown. In either case, if mature lymphocytes come into contact with antigen in the absence of the second signal, the cell enters a prolonged period of unresponsiveness.

considerable fraction of normal splenic B cells have this phenotype.

Thus, antigen-induced unresponsiveness of mature B cells could be an important mechanism for decreasing autoantibody production. Previous studies have demonstrated that clonal deletion or anergy of immature B cells acts on higher affinity B cells but not on lower affinity B cells[11]. If the same is true of clonal anergy of mature B cells, then the only B cells capable of producing autoantibodies would be those of low affinity. Most of the time such low-affinity antibodies are probably not destructive, although there may be exceptions, such as the autoantibodies produced in rheumatic fever.

Mature helper T cells can also become unresponsive on coming into contact with antigen. There are now several reports of situations where the immune system tolerates allogeneic class II MHC molecules (which are recognized by helper T cells) introduced by grafts or tissue-specific expression of a transgene[9,12,13]. Clonal deletion of immature T cells probably cannot operate on T cells recognizing allogeneic MHC molecules unless those molecules are expressed on cells in the thymus (see ref. 9). So acceptance of these tissues probably occurs in the presence of helper T cells that can recognize the antigen[9,12,14]. Similarly, adult Mls-1^b mice made unresponsive to Mls-1^a spleen cells were found to have normal numbers of helper T cells recognizing Mls-1^a, as detected with anti-$V\beta6$ antibodies[15]. The T cells from unresponsive mice were atypical in that they did not proliferate or secrete interleukin-2 in response to stimulation with Mls-1^a spleen cells *in vitro*. Thus, it would appear that unresponsiveness is accompanied by antigen-specific helper T cells existing in an anergic state.

Why do these mature helper T cells fail to initiate graft rejection? One possibility is that the cells recognize antigen in such a way as to induce clonal anergy rather than clonal reactivity. Moreover, tolerance induction of mature helper T cells would go a long way towards explaining tolerance to self-components with restricted tissue distribution, and such a phenomenon has been observed *in vitro* with cloned helper T cells. If these T cells encounter their antigen–MHC complex in a sterile setting, for example in artificial planar membranes or on the surface of lightly fixed antigen-presenting cells, then a profound state of unresponsiveness to antigen is induced[16]. Interestingly, such cells proliferate vigorously on addition of the growth factor interleukin-2, raising the possibility that anergy may be a reversible state. The difference between induction of anergy and induction of a positive response, including lymphokine secretion, is the provision of a second signal (currently unknown) by the antigen-presenting cell.

One thing that remains unclear is what determines whether or not the antigen-presenting cell delivers the second signal to the helper T cell. Recently, transgenic mice were created in which expression of the I–E class II MHC molecule was restricted to the pancreas and kidney[12]. The mice did not reject these tissues immunologically, but the presence of reactive helper T cells could be demonstrated on removal and analysis *in vitro*. This could be an example of clonal anergy induced in mature T cells. In agreement with that idea, I–E-expressing cells from the pancreas were able to induce unresponsiveness in helper T-cell clones, *in vitro*[17]. Perhaps the pancreatic cells lack the capacity to provide the second signal needed by the helper T cells to respond. This suggests that helper T cell recognition of antigen plus MHC on tissue cells may lead to unresponsiveness, whereas more specialized antigen-presenting cells (macrophages, dendritic cells or B cells) may be needed to induce activation.

More experiments will be needed to establish whether the clonal anergy seen *in vitro* with helper T-cell clones is also important *in vivo*. Experiments with transgenic T cells, following the strategy employed by Goodnow *et al.* with B cells, would be one approach. MHC alloantigen expressed on selected tissues may not be a good model for tissue-specific antigens, however, and more experiments are needed with non-MHC antigens expressed in a tissue-specific manner (as has been done with transgene-encoded SV40 T-antigen[18]).

It may be apparent to the reader that there is an underlying similarity between antigen-induced unresponsiveness of cloned helper T cells and that of B cells. In both cases, there are two possibilities for the cell that encounters antigen. In the presence of a second signal coming from a helper T cell or from an antigen-presenting cell, the cell becomes activated; in the absence of the second signal, the cell becomes anergic (see figure). Antigen contact is never neutral. Everything is friend or foe, and the second signal is used to identify the foe. The idea that antigen in the absence of a second signal induces unresponsiveness was originally advanced to explain tolerance nearly 20 years ago by Bretscher and Cohen[19]. The experiments have finally begun to catch up with them. ☐

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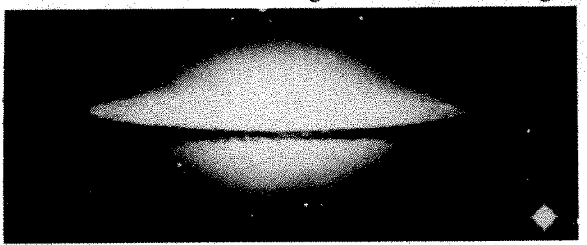
1. Goodnow, C.C. *et al. Nature* **342**, 385–391 (1989).
2. Owen, R.D., Davis, H.P. & Morgan, R.F. *J. Hered.* **37**, 291–297 (1946).
3. Nossal, G.J.V. *A. Rev. Immun.* **1**, 33–62 (1983).
4. Kisielow, P. *et al. Nature* **333**, 742–746 (1988).
5. Sha, W.C. *Nature* **336**, 73–76 (1988).
6. Goodnow, C.C. *et al. Nature* **334**, 676–682 (1988).
7. Nemazee, D.A. & Burki, K. *Nature* **337**, 562–566 (1989).
8. Kappler, J.W., Roehm, N. & Marrack, P. *Cell* **49**, 273–280 (1987).
9. Schwartz, R.H. *Cell* **57**, 1073–1081 (1989).
10. DeFranco, A.L. *A. Rev. Cell Biol.* **3**, 143–178 (1987).
11. Riley, R.L. & Klinman, N.R. *J. Immun.* **136**, 3147–3154 (1986).
12. Nossal, G.J.V. *Science* **245**, 147–153 (1989).
13. Mueller, D.L. *Nature* **339**, 513–514 (1989).
14. Lo, D. *et al. Cell* **53**, 159–168 (1988).
15. Rammensee, H.–G., Kroshcewski, R. & Frangoulis, B. *Nature* **339**, 541–544 (1989).
16. Mueller, D.L., Jenkins, M.K. & Schwartz, R.J. *A. Rev. immun.* **7**, 445–480 (1989).
17. Markmann, J. *et al. Nature* **336**, 476–479 (1988).
18. Adams, T.E., Alpert, S. & Hanahan, D. *Nature* **325**, 223–228 (1987).
19. Bretscher, P. & Cohen, M. *Science* **169**, 1042–1049 (1970).

ASTRONOMY

Galactic disk loses weight

M. G. Edmunds

ASTRONOMERS are so embarrassed these days by finding evidence of hidden mass almost everywhere they look, that it is a refreshing change to read a triplet of papers[1] that implies that, although the existence of dark matter in galaxies still seems inevitable, it is no longer necessary to invoke its presence as a major constituent of the disk of our own Galaxy.



Sombrero galaxy with dense, bright disk and inner spheroid.

The dynamics of stars and gas in the Galaxy are governed by its gravitational field, the structure of which is set by the distribution of mass. Three major components of mass can be identified. From visual and radio observation we know a great deal about the disk of stars and interstellar gas, which rotates inside a spheroid of stars (see figure). The third, invisible, component is the corona (sometimes called the dark halo) whose presence is inferred only by its gravitational influence on the rotation of the disk.

By looking out parallel to the galactic rotation axis from our peripheral position in the galactic disk, one sees a change in number density and velocity dispersion of stars as a function of distance from the plane, which can be used to investigate the gravitational field. The greatest influence in this direction is the mass in the disk. In contrast, the variation of rotation velocity in the disk with distance from the centre (the rotation curve) is dominated by the spheroidally distributed mass of the spheroid and corona.

Kuijken and Gilmore[1] have extensively modelled the stellar density and velocity data perpendicular to the plane, to arrive at a value for the integrated surface mass density in a column through the galactic disk at the Sun. The mass in the disk determined in this way (by the dynamics of stars perpendicular to the galactic plane) is sometimes known as the Oort limit, after the pioneering investigations[2] of the Dutch astronomer Jan Oort. Other investigations have indicated about 50 per cent more mass detected dynamically than could be seen in stars or gas. The new work fits models to the data in a rather more general way, and also takes account of the (previously neglected) effect of the increasing influence of the spheroidal gravitional field on stars that reach high above the plane. A correction is also applied in estimating the number of stars at different heights to take account of the effects of chemical composition of stars on their brightness. Because stars that are far from the disk have, on average, a lower abundance of heavy elements than those close to the disk, neglect of this difference could lead to systematic errors in estimating distances and space densities.

The result of the investigations is a local surface density of 46±9 solar masses per unit area (square kiloparsecs) of disk. This is considerably smaller than previous values. The authors give an estimate in the same units of visible matter in the disk as 35±5 for stars and 13±3 for gas. This totals 48±6, in excellent agreement with the dynamical mass. Thus, although some 10 units of dark matter could still be lurking in the disk without offending the error limits, the importance of this investigation is that it implies there is no need to invoke any dark matter in the disk.

The low limit on disk mass has other implications. The rotation curve from the Galactic Centre to about 50 per cent beyond our radius from the centre is reasonably well determined and can be explained only if there is a dark massive corona. Models have been constructed that try to dispense with the corona but still account for the rotation curve by putting as much mass as possible into the disk and visible spheroid — so called 'maximal disk' models[3]. The new local disk mass limit means that the disk itself cannot be massive enough. Although still not constrained as much as some people would like to believe, the visible spheroid probably cannot provide sufficient mass either.

The rotation curve beyond one-and-a-half times our galactocentric radius is less well measured, but certainly implies a dominant distribution of mass unlike that of the spheroid. The reasons for the uncertainty in spheroid mass are both controversy over its shape, and also because it is difficult observationally to measure its density. The problem arises because there are very few spheroid stars in the local solar neighbourhood where they are overwhelmingly outnumbered by disk stars, although the overall spheroid could still contain a lot of stars in total because of its large volume.

The elimination of dark matter from the disk is a pleasant simplification, because we now have only to worry about the corona, a problem well-known in external spiral galaxies. But the shape and other properties of the corona remain obscure, although Kuijken and Gilmore argue that significant flattening of the corona can be ruled out as it would tend to reduce their dynamical estimate of disk mass below that actually visible in stars and gas. One useful additional constraint they point out is that the modelling of dynamics perpendicular to the disk effectively rules out any model that dispenses with a corona and explains the rotation curve by a modification of the law of gravitation to include an extra source of acceleration parallel to the newtonian force. □

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1. Kuijken, K. & Gilmore, G. *Mon. Not. R. astr. Soc.* **239**, 571–603, 605–649 & 651–664 (1989).
2. Oort, J. *Bull. Astr. Inst. Netherlands* **6**, 249 (1932).
3. Sellwood, J. A. & Sanders, R. H. *Mon. Not. R. astr. Soc.* **233**, 611–620 (1988).

PALAEONTOLOGY

Ears and vertebrate evolution

Alec L. Panchen

IN 1987 the members of a highly successful Anglo–Danish expedition returned from east Greenland with about three-quarters of a tonne of rock matrix containing Upper Devonian fossil tetrapods (land vertebrates)[1]. East Greenland is the only area in the world to have yielded tetrapods of this early date (about 360 million years old) in substantial quantities, but most of the material previously collected is attributed to the genus *Ichthyostega*[2]. The majority of the specimens from the recent expedition appear to be of the very different genus *Acanthostega*, which was formerly known only from a handful of specimens. On page 425 of this issue Clack[3], an organizing member of the expedition, describes the stapes of *Acanthostega* in a first account of the new material. She proposes that the stapes in this very early tetrapod was concerned not with the transmission of air-borne sound but with respiratory movements through a spiracle, as in some fishes. This hypothesis adds a further twist to the seemingly endless controversy over the evolution of hearing in tetrapods.

In most living tetrapods the stapes conducts air-borne sound from the tympanum (eardrum) to the inner ear. In three tetrapod groups — frogs, living reptiles and birds — the stapes is inserted in the tympanum and is the only ear ossicle. It is generally agreed that it is the homologue, at least in part, of the hyomandibula of fishes. In fishes, the hyomandibula, a part of a gill arch, serves to suspend the jaw articulation from the braincase on each side of the skull. In mammals, the middle ear contains not one ear ossicle, but a chain of three (stapes, incus and malleus), the last of which is inserted into the tympanum. Discussion of the homologies of these three ossicles started well before the publication of Darwin's *Origin of Species*, when, in 1837, Reichert showed from embryological evidence that the incus and malleus were the homologues of the cartilages that form the jaw joint in all other jawed vertebrates[4]. Reichert's theory was later interpreted in evolutionary terms and is corroborated by evidence from the fossil forebears of mammals[5]. This theory is generally (but not universally[2]) accepted today.

It also seems certain, however, that the nearest common ancestor of mammals on one hand, and reptiles and birds on the other, did not have a tympanic ear. In those fossils believed to be close to that common ancestor (that is, the most primitive amniotes) the orientation of the stapes is like that of the fish hyomandibula from which it was derived, and there are no signs of the characteristic otic notches at the back of the skull that held the tympanum in other early tetrapods. Furthermore, there is evidence that the tympanic ear of frogs evolved separately from that of any amniote[6]. Thus a tympanic ear must have evolved convergently in three unrelated groups of tetrapods — frogs, reptiles and birds, and mammals.

Frogs, and more doubtfully other extant amphibia, are members of a larger group of primitive tetrapods, the temnospondyls. Most fossil temnospondyls have a pair of otic notches at the back of the skull roof and a lightly built stapes directed towards each notch. It is thus probable that they had tympanic ears. But the most primitive temnospondyls, notably *Greererpeton* from the Carboniferous period, had a massive stapes with a skull-bracing function[7]. Clack has shown that another Carboniferous tetrapod, *Pholiderpeton*, which is thought to be related to the amniote line, has a strikingly similar stapes[8]. Both *Greererpeton* and *Pholiderpeton* lack otic notches.

Clack now shows[3] that the stapes of the much earlier *Acanthostega* is of the same type. Yet *Acanthostega* also appears to have otic notches. She suggests, however, that these did not hold tympana, but that each formed the margin of an open spiracle. She further suggests that the stapes was concerned with pumping movements to draw in either air or water through the spiracle for respiration. In fish where the spiracle remains open, notably in skates and rays, the hyomandibula helps to pump water into the buccal cavity; the water is then expelled through the other gill slits. Clack suggests that the stapes of *Acanthostega*, the homologue of the hyomandibula, also had this respiratory function, but probably to ventilate the lungs.

If Clack and her colleagues had not recovered stapes associated with skull material, one might have predicted that the function of *Acanthostega's* stapes was to conduct sound from a tympanum held in the otic notch. Given that three unrelated tetrapod groups are known to have this arrangement, it would have been reasonable to suppose that this was the primitive tetrapod condition and thus would be found in *Acanthostega*. The lack of otic notches, and the massive stapes found in *Greererpeton* and *Pholiderpeton*, could then have been seen as secondary features. The discovery in *Acanthostega* of both massive stapes and otic notches thus forces a re-evaluation of the primitive tetrapod condition that will inevitably affect our ideas about tetrapod phylogeny. ☐

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1. Panchen, A.L. *Nature* **333**, 704 (1988).
2. Jarvik, E. *Basic Structure and Evolution of Vertebrates* (Academic, London, 1980).
3. Clack, J.A. *Nature* **342**, 425–427 (1989).
4. Goodrich, E.S. *Studies on the Structure and Development of Vertebrates* (Macmillan, London, 1930).
5. Kemp, T.S. *Mammal-like Reptiles and the Origin of Mammals* (Academic, London, 1982).
6. Lombard, R.E. & Bolt, J.R. *Biol. J. Linn. Soc.* **11**, 19–76 (1979).
7. Smithson, T.R. *Zool. J. Linn. Soc.* **76**, 29–90 (1982).
8. Clack, J.A. *Zool. J. Linn. Soc.* **79**, 121–148 (1983).

AMAZON DEFORESTATION

Predicting climate effects

Robert E. Dickinson

FEARS that global and local meteorological balances might be upset by the deforestation of the Amazon have made the destruction of the rainforest a matter of wide public concern. Elsewhere in this issue (*Nature* **342**, 411–413; 1989), J. Lean and D. Warrilow describe detailed computer simulations that reveal the impact that might be expected if large tracts of rainforest are replaced by pasture. Most strikingly, they find that rainfall in the region could be dramatically reduced.

The role of tropical forests in the global climate system has long been a subject of speculation. Brazilian studies of the hydrological balance in the Amazon have done much to foster the debate over the past decade. Joint UK–Brazilian micrometeorological investigations are now giving us a much clearer view of the balances of energy and water in and above the forest canopy. But it is only with detailed climate models, such as those used by Lean and Warrilow and also by C. Nobre, J. Shukla and P. Sellers (submitted to *Science*), including representations of the role of vegetation in the water and surface-energy balances, that we can make quantitative estimates of the regional and global changes that may follow extensive deforestation.

As well as predicting a 20 per cent decrease in rainfall over the Amazon region, Lean and Warrilow suggest that the local climate will become warmer and that evapo-transpiration will be reduced. My earlier study with A. Henderson-Sellers and M.F. Wilson (see *Q. Jl R. met. Soc.* **114**, 439–462; 1988), using the National Center for Atmospheric Research community climate model (CCM), also indicated that warming and reduction of evapo-transpiration on a similar scale would follow deforestation, but the model hydrological cycle was too 'noisy' to establish any clear indication of changes in rainfall.

Regional rainfall in the Amazon is highly variable, in both space and time, owing to variations in tropical ocean temperatures. Until climate models can reproduce this variability, projections of changes induced by altered land use will be greeted with scepticism. The hydrological cycle is regarded by many as the weakest component in climate models. The main difference between our earlier study and Lean and Warrilow's is in the numerical treatment of the spatial dimensions, their approach avoiding hydrological difficulties associated with ours. The link the new study seems to have established between rainfall and tropical forests is one of the strongest of its kind yet modelled.

The suggestion of this link increases the urgency with which related questions must be addressed. How do the rainfall anomalies affect weather patterns in the Northern Hemisphere? By analogy with the El Niño–Southern Oscillation phenomena, large-wavelength, transverse 'Rossby' waves in the atmosphere might propagate long-range effects. Will the accompanying changes in ocean temperatures amplify or mitigate the predicted effects? It will be important to find out whether climate changes due to deforestation will act through the forest ecosystem to accelerate the current decline. It may be that the tropical forests are sufficiently stable that they will grow back once human intervention ceases.

The interest in the rainforest reminds us of the other effect of vegetation on climate. Vegetation is a reservoir for carbon that annually exchanges with the atmosphere an order of magnitude more CO_2 than is added by human activity. The loss of the tropical forests is recognized as adding significantly to the increases in atmospheric CO_2. As the political will grows to prevent such increases, it is

100 years ago

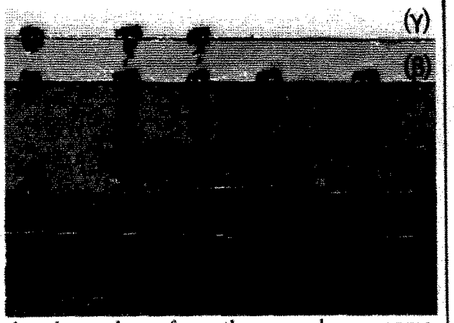
Mirage in the South American Pampas

I WAS staying in the Pampas of the Argentine Republic, near Melincue, a small town of the Provence of Santa Fe, from September 1888 to March 1889. During my stay I had the opportunity of observing certain mirage phenomena. To illustrate my observations I had drawn eight diagrams; but, for the purpose of insertion in *Nature*, I have been obliged to reduce these. Hence I fear that my descriptions may not be as clear as I should wish.

The Winter Mirage.

I saw this mirage several times, always about sunrise and after a frost. There would be, for example, just below, or on the edge of the line (α), a cow. This I will call the *"first cow,"* or the "original cow." Just below or on the line (β), vertically above the *first cow*, and, like it, erect, would be a *second cow*, a repetition of the first. And often, above this again, below or on the line (γ), would be a third cow, also erect.

Sometimes there were confused images hanging from the *second cow* and joining other confused images piled on the *first cow*; sometimes the first cow was clear of images, while



they hung down from the second cow; sometimes the second cow was near, and there were images piled on the first.

The mirage lasted until about an hour and a quarter after sunrise. W. LARDEN

From *Nature* **XLI**, 69–71; November 1889.

worth noting that the cost of techniques for reducing CO$_2$ emissions, up to many hundreds of dollars per tonne of carbon removed, probably outweighs any economic benefit gained from forest destruction. It is easy to imagine that the path with least initial cost to reduce CO$_2$ inputs to the atmosphere would be in the form of payments to countries with tropical forests for a reduction in cutting and burning.

The many political, social and ecological factors behind tropical deforestation make this a complex issue. But although studies of the climatic impact of deforestation will help climate modellers understand the important features in the weather system, it is questionable that they will help policy makers in tropical countries tackle their ecological problems. ☐

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MID-OCEAN RIDGES

Mixing of basalt magmas

Emily M. Klein

THE pressure at which melting and melt segregation occur to produce mid-ocean ridge basalt (MORB) magmas is at the heart of a long-running controversy. This is due to the fact that from the pressure one can constrain physical characteristics of divergent plate boundaries, such as the temperature structure beneath the ridge axis or the width of the melting zone, and chemical characteristics of the primary magmas in equilibrium with the mantle source. Some argue[1] that primitive MORBs represent nearly primary magmas in equilibrium with the mantle at pressures as low as 7 kbar, corresponding to a relatively shallow depth, 21 km, beneath the ridge axis. Others suggest[2] that extensive crystallization *en route* to the sea floor has substantially modified the primary melt that was generated at pressures of 15–30 kbar (45–90 km). Although the two schools may seem irreconcilable, Salters and Hart, on page 420 of this issue[3], contribute to a growing body of evidence suggesting that advocates of both extremes may, in fact, be correct, united under a more realistic model for the melting process beneath ocean ridges.

In their study, Salters and Hart examine Lu–Hf and Sm–Nd isotope and trace-element systematics in MORB and ocean-island basalts. The authors identify a discrepancy between the measured parent-to-daughter trace-element ratios of Sm/Nd and Lu/Hf, on the one hand, and their isotope ratios ^{143}Nd/^{144}Nd and ^{176}Hf/^{177}Hf, on the other. The observed isotope ratios are 'unsupported' in the sense that the high ratios imply parent-to-daughter trace-element ratios higher than observed. For the Lu–Hf system, the authors term this discrepancy the Hf-paradox and explore the possibility that the decoupling between trace-element and isotope ratios occurs during the melting process itself.

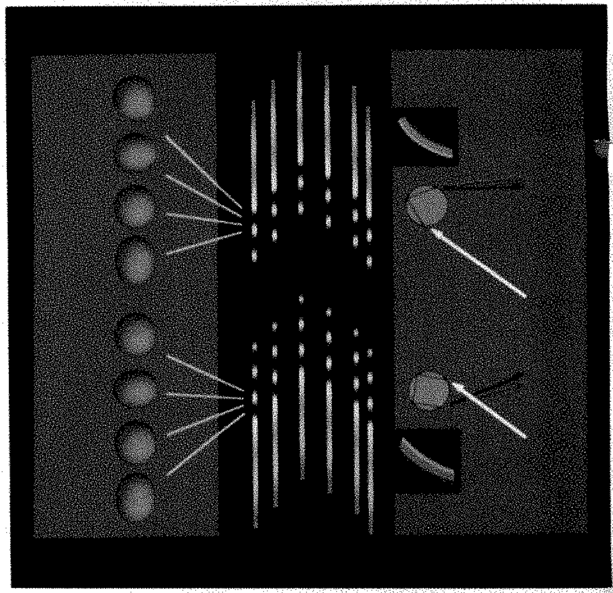
Specifically, if garnet is a residual phase during the melting process beneath the ocean ridge, the greater chemical affinity of garnet for Lu than for Hf will lead to low Lu/Hf ratios in the melts produced within the garnet stability field. The stability of garnet as a residual phase in turn implies that a significant portion of the melting process occurs at pressures greater than about 20 kbar, where garnet replaces spinel as the stable aluminous mantle phase.

Salters and Hart adopt a more realistic melting model that recognizes that if melting occurs by adiabatic decompression, it must be polybaric, with melts generated (and segregated) over a range of pressures beginning deep within the garnet stability field and extending to lower pressures within the spinel stability field. The basaltic magma erupted at the ridge axis thus represents an average composition generated by the pooling of melts from throughout the polybaric melting column. From this perspective, advocates of high- or low-pressure melting are both correct in the sense that melting occurs over a range of pressures. It is important to recognize, however, that for a pooled melt composition, the concept of a single pressure of equilibration during melting, as implied in the earlier studies, has no meaning.

Studies of the major-element compositions of MORB also support the concept of a polybaric melting column and the conclusion that for the majority of MORB magmas, melting begins at high pressures (20–40 kbar). Examining the range of major-element compositions observed among regional averages of MORB chemistry, Langmuir and I noted[4] that parameters indicative of the extent of melting, such as sodium content or the ratio of calcium to aluminium, correlate with parameters indicative of the pressure of melting, such as iron or silica.

As with the Lu–Hf systematics, this relationship can most easily be understood in terms of the pooling of melts from throughout polybaric melting columns; the height of each column is governed by the solidus pressure and therefore the mantle temperature beneath the ridge axis for a given region. The observed correlation between the extent of melting and pressure of melting results from the fact that the deeper the intersection of the mantle solidus, the greater the proportion of melt generated at high pressure and the greater the total amount of melting achieved. Indeed, Salters and Hart note the complementary nature of the major-element, trace-element and isotope data and observe that their indicator of the pressure of melting, δ(Lu/Hf), correlates

Rainbows reveal good vibrations



How big is a raindrop? One way to find out is to use radar. Air pressure flattens falling drops, more so for larger drops, the oblateness being detectable with polarized radar beams. But, as Kenneth Beard and colleagues point out on page 408, oscillations of the raindrops, by averaging effects can make them seem smaller. This effect becomes noticeable for drops 1–1.5 mm in diameter, possibly owing to a resonance between the natural frequency of drops this size (a few hundred hertz) and the frequency with which air eddies in the wake are shed. To test this hypothesis, Beard *et al.* observed the changing rainbow reflections of white light scattered by each drop as it fell. Lamp and detector are arranged to favour red light reflections. As the drop oscillates, either yellow or infrared (dark) light is preferentially reflected, leaving a characteristic pattern on streak photographs of the falling drops, as depicted above (top, primary rainbow; bottom, secondary). ☐

with major-element indicators of the extent of melting, such as sodium.

The idea that the generation of MORB involves the pooling of melts is also supported indirectly by a study of Sr, Nd and Pb isotopes in basalts recovered from near-ridge seamounts and their adjacent ridge segments, reported by Castillo and Batiza in *Nature* last week[5]. Previous studies of the East Pacific Rise axis and adjacent seamount isotope systematics have shown that, although the two environments overlap in isotope composition, the isotope diversity of the seamount lavas is considerably greater than that of the ridge-axis lavas[6]. The proximity of the ridge and seamounts suggests a similar mantle source, composed of dispersed, isotopically heterogeneous mantle material.

The greater isotopic diversity of the seamounts may therefore result from the sampling of isotopically distinct reservoirs and eruption of the distinct magma compositions, while the greater homogeneity of the ridge-axis magmas may result from the pooling of these isotopically diverse melts before eruption. This interpretation is easy to fathom for the fast-spreading Pacific ridges, where robust magma supply and the presence of a subaxial magma chamber[7] can be expected to foster homogenization of diverse melt compositions.

Castillo and Batiza show that the relationship observed in the Pacific also holds for seamounts and the adjacent segment of the mid-Atlantic ridge in the South Atlantic. This is despite the fact that the spreading rate of the southern mid-Atlantic ridge is one-quarter that of the East Pacific Rise, that magma supply is presumably less voluminous, and that evidence of a steady-state magma chamber to facilitate homogenization has yet be documented. Nevertheless, the isotope study of Castillo and Batiza suggests that significant mixing of isotopically distinct melts occurs even beneath the mid-Atlantic ridge, attesting to the homogenization of melts even at slow spreading rates. Although it is not clear where this homogenization process is taking place, whether in magma conduits or perhaps in small, ephemeral magma chambers, there is now a substantial body of evidence from isotopes, trace elements and major elements that MORB-magma genesis involves the pooling of melts from a heterogeneous mantle over a wide range of pressures. ☐

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1. Presnall, D.C. & Hoover, J.D. *Contrib. Mineral. Petrol.* **87**, 170–178 (1984).
2. O'Hara, M.J. *Nature* **220**, 683–686 (1968).
3. Salters, V.J.M. & Hart, S.R. *Nature* **342**, 420–422 (1989).
4. Klein, E.M. & Langmuir, C.H. *J. geophys. Res.* **92**, 8089–8115 (1987).
5. Castillo, P. & Batiza, R. *Nature* **342**, 262–265 (1989).
6. Zindler, A., Staudigel, H. and Batiza, R. *Earth planet. Sci. Lett.* **70**, 175–195 (1984).
7. Detrick, R.S. *et al. Nature* **326**, 35–41 (1987).

ARCHAEOLOGY

Age and the female form

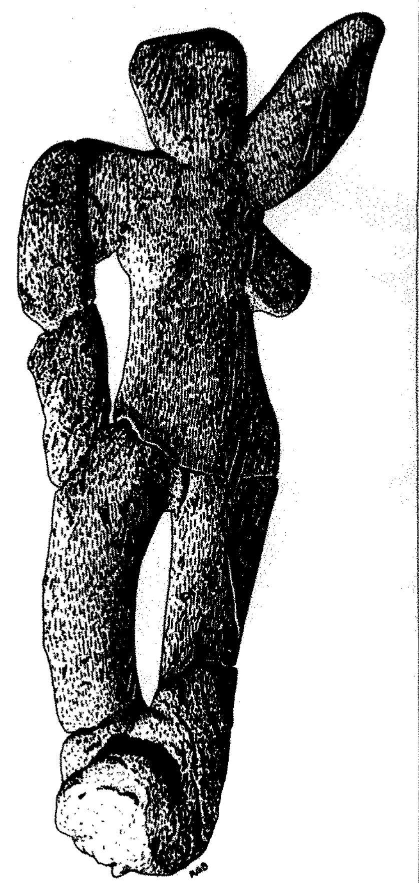
Paul G. Bahn

ARCHAEOLOGISTS in Austria have discovered one of the world's earliest known sculptures, in the form of the oldest dated female figurine[1]. The find is remarkable not merely for its antiquity but also for the artistic sophistication it displays.

Since 1941 the loess sediments of the Galgenberg site, near Krems, have been known to contain bones of Ice Age mammals (including reindeer and mammoth) together with abundant charcoal and flint flakes. New excavations in a nearby vineyard, directed by Christine Neugebauer-Maresch for the Österreichisches Bundesdenkmalamt, led to the discovery in September 1988 of the fragments of the female figurine. Charcoal samples from the same layer have produced radiocarbon dates of 29,200 to 31,190 years before present (BP).

The 'Dancing Venus of Galgenberg', as the sculpture has been dubbed, is 7.2 cm long, and carved in green serpentine. Unlike the well-known 'Venus figurines' of the Ice Age, such as that from Willendorf in Austria (found almost exactly 80 years earlier to the day), the Galgenberg specimen is flattish rather than carved in the round; its shape was probably predetermined by the stone used, because serpentine often occurs in slabs. The figure has not been polished, and its surface shows the marks of tools and erosion.

The right arm and the legs are supported at both ends, while the left arm appears to be folded back at the elbow. Body weight is supported primarily on the left leg, while the right leg rests on a slightly higher support. The right hand is placed on the hip. This pose causes the left breast to be depicted almost in profile, while the right is in very low relief because of the stone's flatness. The vulva is marked (a feature rare in Ice Age depictions of females[2]), and in further contrast to many of the better known 'Venus figurines' there is no hint of obesity or of an emphasis on breasts and buttocks. The figure is more or less anatomically accurate, except for the thickened limbs which are probably as thin as the artist dared carve them without making the sculpture unduly fragile.



The 'Dancing Venus of Galgenberg', which is some 30,000 years old. The sculpture is 7.2 cm long and is carved from green serpentine stone. (Drawing by R. Bednarik, from a photograph.)

There are two important points about the Galgenberg figurine. First, it is at least 5,000 years older than the 'Gravettian period' to which most 'Venus figurines' are usually, often unjustifiably, assigned; most of the Western European specimens have no archaeological context whatsoever, and are merely assumed to be Gravettian[3], whereas dated specimens in Eastern Europe in fact span a period from about 23,000 to 12,000 years BP[4].

Second, the Galgenberg carving is the result of considerable technological skill — the stone is rather delicate and brittle, and the head, left arm and breast could all easily have broken off. In addition, the two openings (under one arm and between the legs) would have required a delicate boring operation; the later 'Venus figurines' are normally static, solid symmetrical figures with no perforations and no protruding limbs[5].

The sophistication revealed in both technology and composition can only be the end-result of a long tradition in carving (perhaps of perishable materials). The same conclusion had already been reached from study of the contemporary or even older ivory carvings of animals and humans from West Germany (Vogelherd, Geissenklösterle, Hohlenstein-Stadel)[6] and perhaps also the ivory human figurines from Brassempouy in France[7].

These objects, the oldest figurative carvings known at present, simply cannot represent the 'first art', which must therefore have appeared long before.

In this respect, the Acheulian female 'figurine' from Berekhat Ram, Israel[8] may provide a crucial clue. Dating to between 230,000 and 800,000 years BP, it is a small scoria pebble whose shape, naturally resembling a 'Venus figure', may have been modified by several grooves. Whether or not it was modified, its very presence at Berekhat Ram implies that the occupants of the site recognized its iconic properties, and therefore that an interest in the female form is one of the most enduring features of mankind. ☐

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1. Bednarik, R.G. *Rock Art Res.* **6**, 118–125 (1989).
2. Bahn, P.G. *Rock Art Res.* **3**, 99–105 (1986).
3. Bahn, P.G. & Vertut, J. *Images of the Ice Age* 85 (Windward, Leicester/Facts on File, New York, 1988).
4. Soffer, O. in *The Pleistocene Old World* (ed. Soffer, O.) 335 (Plenum, New York, 1987).
5. Delporte, H. *L'Image de la Femme dans l'Art Préhistorique* (Picard, Paris, 1979).
6. Marshack, A. in *L'Homme de Néandertal: 5, La Pensée* (ed. M. Otte) 57–91 (ERAUL, Liège, 1988).
7. Bahn, P.G. & Vertut, J. *Images of the Ice Age* 140 (Windward, Leicester/Facts on File, New York, 1988).
8. Goren-Inbar, N. *Mi'Tekufat Ha'Even* **19**, 7–12 (1986).

CHEMICAL PHYSICS

Size-selected solvent cages

Tony Stace

AN isolated diatomic molecule photoexcited to an electronic state above the dissociation limit will swiftly break up into free atoms (Fig. 1). In a liquid or dense gas, however, the relative kinetic energy of the excited atom pair is often lost to a surrounding solvent cage before it can separate. This is the cage effect, first proposed by Franck and Rabinowitsch[1] to explain the reduced photochemical yield, ϕ, of free radicals (atoms or molecular fragments with an unpaired electron) in solution. Attempts in liquid-phase experiments[2] to reveal details of the process at a molecular level are impeded by the bulk physical properties of the liquids. In an effort to resolve this problem, Lineberger and colleagues[3] have studied the dynamics of atom recombination (caging) in mass-selected cluster ions.

Small clusters comprising a chromophore, I_2^- (which absorbs light at a wavelength of 720 nm) and 1–20 carbon dioxide molecules in the form $I_2^-.(CO_2)_n$, generated in a vacuum at the entrance to a mass spectrometer, are mass-selected and excited by a pulsed dye laser. Depending on the value of n, this induces either of the following photofragmentation steps:

$$I_2^-.(CO_2)_n \xrightarrow{\text{light}} I^-.(CO_2)_m + I + (n-m)CO_2 \quad (1)$$

$$\xrightarrow{\text{light}} I_2^-.(CO_2)_k + (n-k)CO_2 \quad (2)$$

In reaction (1), I^- has photodissociated in much the same way that it might as an isolated gas-phase species. But as n increases, there is a tendency for reaction (2) to dominate: under those circumstances, the CO_2 molecules are operating as an effective cage. They absorb the kinetic energy of the separating fragments, and dissipate it by being evaporated from the cluster[4]. Provided the energy of I_2^- falls below the bond energy for the ground state (Fig. 1), the molecular ion is stabilized and cannot dissociate. Plotted in Fig. 2 is the product fraction resulting from reaction (2) as a function of cluster size; in effect, the graph is a plot of $1 - \phi$. As might be expected, increasing the size of the cluster increases the probability of caging. In previous experiments, Lineberger's group[5] showed that, for a given size of cluster, the photofragment yield, ϕ, increases with photon energy.

Since the early work by Franck and Rabinowitsch[1], there have been many attempts to characterize the cage effect in terms of both timescale and molecular or solvent parameters. These efforts were limited by the fact that it was not possible to monitor the influence of a single solvent variable: changing the solvent molecular mass invariably leads to a change in solvent size and/or viscosity. But about 15 years ago Troe and co-workers[2] used simple solvents, such as condensed inert gases, to allow a more systematic variation of parameters; thus a change from liquid argon to liquid xenon gives a considerable difference in mass, but not in viscosity. Also, the photochemical yield could be monitored as a function of gas pressure. In this way, Troe and colleagues spanned the complete range of behaviour, from low pressures for which $\phi \simeq 1$, through to very high pressures (10,000 atmospheres) where little or no photodissociation was observed.

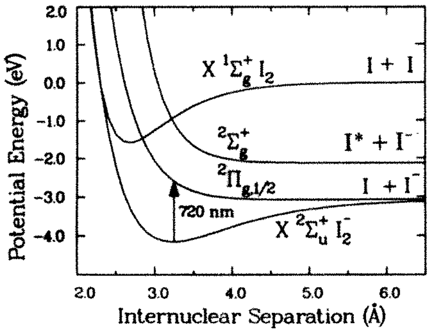

FIG. 1 Approximate potential-energy curves for I^- and I_2. In the clusters the $^2\pi_{g,1/2} \leftarrow {}^2\Sigma_u^+$ transition between the bound ground state and a low dissociating state is excited with 720 nm laser radiation.

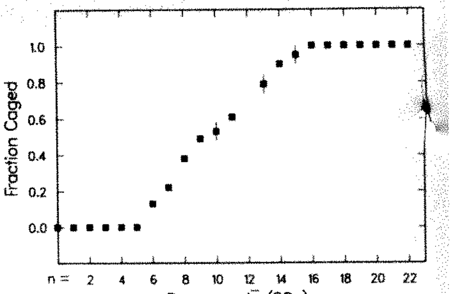

FIG. 2 Fraction of products resulting from reaction (2) as a function of cluster size, *n*, following the photoexcitation of $I_2^-.(CO_2)_n$ with laser radiation of wavelength 720 nm. (Lineberger *et al.*, unpublished results.)

As might be expected, dense Xe, N_2 and CO_2 act as relatively efficient cages, whereas He and Ne do not. At the same time, picosecond (10^{-12} s) time-resolved measurements of the photodissociation of I_2 in liquid by Chuang *et al.*[6] greatly elucidated the dynamics of the caging process. In particular, they led to the development of models for caging based on a diffusion-controlled recombination of geminate (original) pairs.

The current experiments by Lineberger and co-workers can be seen as a natural step forward from both these techniques. The group seeks to take an individual localized environment, as seen by the excited molecule or ion, and establish how effective it is at promoting a cage event. The latest experiments are time-resolved, and yield a measure of how quickly ground-state I_2^-, in reaction (2), recovers its original electronic and vibrational state. The measured time, just 10 ± 5 ps for $I_2^-.(CO_2)_{16}$ (about 500 CO_2 vibrational periods), is a convoluted response derived from both the experimental situation and instrumental parameters.

On a picosecond timescale, there are several factors associated with the physics of photodissociation and recombination that could influence events: curve-crossing (electronic-to-translational energy exchange) from the excited electronic state to the final ground-state; vibrational relaxation of the ground state (the electronic absorption profile of I_2^- will change according to its vibrational energy content); and the possibility of recombination into one of several low-lying electronic energy states (a complete description of the electronic states of I_2^- is more complex than that given in Fig. 1). Taking into consideration the results depicted in Fig. 2, one might conclude that it requires 12 or more molecules to establish an effective cage, and that under those circumstances, recombination of the iodine atoms is very rapid.

In a liquid, a small chromophore such as I_2^- could have between two and ten immediate neighbours, depending on the size of the solvent molecules. Therefore, the cluster results support the view that

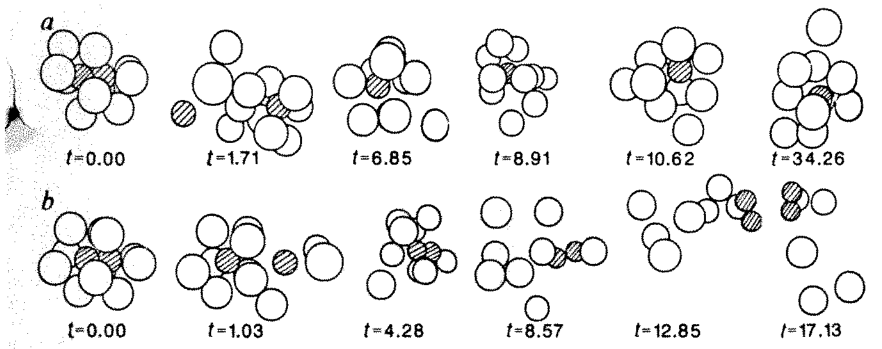

FIG. 3 Results of a computer simulation[7] of events which immediately follow the photoexcitation of $Br_2^-.(CO_2)_{11}$; the bromine atoms are shaded. *a*, The Br_2^- photodissociates; *b*, the cage event leads to the cluster losing most of its carbon dioxide molecules. (Time, *t*, in picoseconds.)

liquid-phase caging[6] is a fast, localized phenomenon, and that most events are probably geminate processes. But a direct extrapolation from cluster to liquid is not necessarily valid: energy flow from a cluster is an irreversible process that has no liquid-phase analogue[3].

Parallel to the experimental progress has been the development of theoretical techniques designed to simulate the caging process at a molecular level[2]. Many of these have evolved from advances in the computer simulation of simple liquids. As one might expect, it is easier to simulate liquid argon than it is liquid benzene or hexane, so that the experiments of Troe and co-workers[2] have provided an ideal source of experimental data. One obstacle associated with the computer simulation of simple liquids remains, namely boundary conditions. The computer, both by virtue of its finite memory and lifetime of the operator, can handle only a limited number of particles — typically 250–1,000 for a physical timespan of about 10^{-10} s. To achieve macroscopic proportions, an 'infinite' three-dimensional lattice is created by defining boundaries that are translated images of the particles under consideration.

The beauty of Lineberger's experiments is that, at least for the simulation of the cage effect, such artificial barriers are no longer necessary. The dynamics of the caging process in a simple cluster, $I_2^-.(CO_2)_n$ or $Br_2^-.(CO_2)_n$, can be followed using numerical techniques to solve Hamilton's classical equations of motion for the complete system. Figure 3 shows two examples where a computer has been used[7] to simulate the sequence of steps which immediately follow the photoexcitation of $Br_2^-.(CO_2)_{11}$ (for the purposes of the present discussion, the behaviour of $I_2^-.(CO_2)_n$ and $Br_2^-.(CO_2)_n$ is identical). At time $t = 0$, the Br_2^- chromophore absorbs a photon which increases the total energy of the cluster by approximately 1.7 eV. The temporal evolution of the cluster is then followed for up to 34 ps, during which time either of reaction steps (1) and (2) can occur. In the simulation depicted in Fig. 3*a* an ion photodissociates, and a Br atom can be seen leaving the cluster after approximately 1.7 ps. In contrast, Fig. 3*b* shows a caging event where the excess energy in the chromophore has been successfully removed, but at the expense of almost all the CO_2 molecules contained in the cluster. The timescale for the latter, 17 ps, is in reasonable agreement with that determined by Lineberger and co-workers.

Computer techniques are available that allow simple molecules, such as N_2 and CO_2, to be treated as structured, rigid, rotating molecules in simulations of this type[8]. Although such a step might seem attractive, it may not help clarify caging. An important relaxation mechanism must be the exchange of energy from the vibrational modes of the excited chromophore to the vibrational modes of the molecules in the solvent cage. Treating the cage molecules as rigid rotors excludes such an exchange. But computer simulation techniques are a long way from being able to treat collections of vibrating molecules. To some extent, the ball is still in the experimentalist's court; theorists now need results for $I_2^-.Ar_n$ or $I_2^-.Kr_n$. □

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1. Franck, J. & Rabinowitsch, E. *Trans. Faraday Soc.* **30**, 120–131 (1934).
2. Schroeder, J. & Troe, J. *A. Rev. phys. Chem.* **38**, 163–190 (1987).
3. Ray, D., Levinger, N.E., Papanikolas, J.M. & Lineberger, W.C. *J. chem. Phys.* **91**, 6533–6534 (1989).
4. Stace, T. *Nature* **327**, 186–187 (1987).
5. Alexander, M.L., Levinger, N.E., Johnson, M.A., Ray, D. & Lineberger, W.C. *J. chem. Phys.* **88**, 6200–6210 (1988).
6. Chuang, T.J., Hoffman, G.W. & Eisenthal, K.B. *Chem. Phys. Lett.* **25**, 201–205 (1974).
7. Perera, L. & Amar, F.G. *J. chem. Phys.* **90**, 7354–7368 (1989).
8. Stace, A.J. *J. chem. Soc. Faraday Trans. 2* **75**, 2105–2110 (1981).

DNA FINGERPRINTS

Victims or perpetrators of DNA turnover?

Gabriel A. Dover

As revolutions go, the discovery of restless minisatellite DNA families (the basis of DNA fingerprinting in humans)[1] is here to stay; and as with all good revolutions it has incited the search for other unruly sequences. As the evidence emerges, it seems that human minisatellite DNA is at the tip of an iceberg of many types of more passively fluctuating repetitive sequences.

It was no accident that Jeffreys called regions of hypervariable length fluctuations 'minisatellite' DNA, because of their similarity in organization to the large blocks of maxisatellite DNAs. The ease of isolation and sequencing of maxisatellites quickly gave investigators a panoramic view of a landscape seemingly populated by every conceivable type of satellite DNA, in terms of repeat-unit length (2–2,000 base pairs (bp)), sequence composition, copy number and chromosomal distribution. Are minisatellites as diverse and bewildering as the maxisatellites and multigene families?

The first analysis of human minisatellite repeating units exposed a conserved 'core' sequence with similarities to the *chi* recombination signal in *Escherichia coli*[1] and probably serving as a hotspot for meiotic unequal crossingover[2]. Is this sequence an obligatory constituent within all DNA families showing hypervariability in copy number? Or are there many (possibly thousands) of repeated DNA families wildly fluctuating as a consequence of unequal crossingover and alternative DNA turnover mechanisms such as slippage, rolling circles, and those responsible for telomeric growth, which seemingly do not require *chi*-like sequences? With a nod to the 'selfish DNA' debate of the innocent early 1980s, we can ask if hypervariable minisatellites are constitutionally selfish or blissfully ignorant?

From comparisons of core sequences of the several minisatellite DNAs listed in the table (see ref. 3 and references therein) it could be that the conserved core sequence (GGCAGGAXG) (dashed underline in the table) found by Jeffreys is not widespread, and, given the G-rich composition of the majority of sequences being compared, the statistical meaning of the one remaining common sequence G-----GG (underlined) is not entirely convincing. Not only is this relic similarity even further removed from the core sequence, but it also places doubt on its functional relationship (if any) to the *chi* recombination signal (GCTGGTGG). Moreover, other AT-rich minisatellites, derived from the apolipoprotein B gene and the collagen type II gene, do not share any core homology with G-----GG or *chi*.

In similar vein, hypervariable length variation within exons can be due both to repeats containing *chi*-like sequences (as in the silk-moth chorion genes[4]) or repeats not containing *chi*-like sequences (as in the human proline-rich protein genes[5]) (see table). The short length of the repeat (9 bp) in the former indicates slippage, whereas the longer repeat (48–63 bp) in the latter indicates unequal crossingover, as mechanisms of turnover.

Some representative 'core' and simple-sequences from hypervariable repeats (see also ref. 3)

G-rich core sequences		
E. coli		
Chi	GCTGGTGG	
Human		
Myoglobin	GGAGGTGGGCAGGAAC	
consensus 'core'	GGNNGTGGGCAGGAXG	
(using myoglobin probe)	————————	
Bird (willow warbler)		
minisatellite — 1	AGGGAAGGGCTC	
minisatellite — 2	GGGGACAGGGGAC	
(using myoglobin probe)		
Human		
zeta globin	GGTTGTGAGTGGGGCACA	
insulin	ACAGGGGTGTGGGG	
Alpha globin	GAGCGACACGGGGG	
Harvey-*ras* oncogene	GGGGGAGTGTGGCGTCCC	
Phage		
M13	GAGGGTGGNGGNTCT	
A-rich core sequences		
Human		
Apolipoprotein B	TTTATAATTAATATTTT	
Collagen type II	CAATATAGAATAATATA	
Simple sequences		
probes used to isolate minisatellites	synthetic	$(CAC)_n$
	human	$(GT)_n$
	Drosophila	$(CAG)_n$
	whale	$(CT)_n$
	Drosophila *per* locus	$(ACNGGN)_n$ and $(TCAGGC)_n$
Hypervariable exonic repeats		
Chi-like?		
Silk-moth chorion genes	TGYGGXGGX	(X = A,T,C) (Y = T,C)
Not *chi*-like?		
Human proline-rich protein genes	. .AGGAAAGCCACAGGACCACCCC. .	

The widespread occurrence and high sequence heterogeneity of slippage-generated simple sequences means that a great proportion of eukaryotic genomes have the potential to yield hypervariable arrays that are unique to one or other single locus, a situation of some importance in the application of DNA fingerprinting to population biology. This has been brought dramatically to the fore by the recent exploitation by several investigators, using the polymerase chain reaction, of naturally occurring, simple sequence arrays of GT repeats from humans, CAG repeats from *Drosophila melanogaster* and CT repeats from a whale, as hypervariable single-locus probes for easy two-band individual identification in the respective species (see ref. 6 and references therein). Synthetic oligonucleotides based on non-*chi*-like ACNGGN and TCAGGC repeats from within the *per* gene of *D. melanogaster* and the synthetic $(CAC)_5$ have also been used successfully as fingerprint probes, indicating that simple sequences are a rich untapped source of fluctuating length variants.

It is becoming increasingly clear that eukaryotic nuclear genomes (including extensive regions of exons and control sequences) are largely non-random and repetitive on both a fine-grained and a larger scale[7]. They are played upon by half a dozen sequence-dependent and sequence-independent mechanisms of turnover. To misquote Tolstoy: all families have their ups and downs, but each suffers in its own way.

Furthermore, all the mechanisms of amplification and turnover frequently operate one on top of another. For example, slippage (and micro gene conversions) may often be churning around within human minisatellite arrays at some loci during spermiogenesis[2], and in arrays of the *Drosophila* ribosomal RNA genes[8], generating complex patterns which are uninterpretable using simplistic models of selection or drift which do not incorporate DNA turnover. There are probably few long tracts of randomly organized single-copy DNA marching to the beat of a 'molecular clock'[9]. Minisatellites, whether victims or perpetrators of turnover, are seemingly the tips of icebergs of hundreds of heterogeneous families, tightly packed together throughout most of the genome. Human genome sequencers beware — the going will be tough. ☐

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1. Jeffreys, A.J., Wilson, V. & Thein, S.L. *Nature* **314**, 67–73 (1985).
2. Jeffreys, A.J., Royle, N.J., Wilson, V. & Wong, Z. *Nature* **332**, 278–281 (1988).
3. Nakumura, Y. *et al. Science* **235**, 1616–1622 (1987).
4. Eickbush, T.H. & Burke, W.D. *J. molec. Biol.* **90**, 357 (1986).
5. Lyons, K.M., Stein, J.H. & Smithies, O. *Genetics* **120**, 267–278 (1988).
6. Tautz, D. *Nucleic Acids Res.* **17**, 6463–6472 (1989).
7. Tautz, D., Trick, M. & Dover, G.A. *Nature* **322**, 652 (1986).
8. Hancock, J.M. & Dover, G.A. *Molec. Biol. Evol.* **5**, 377–391 (1988).
9. Dover, G.A. *J. molec. Evol.* **26**, 47–58 (1987).

DAEDALUS

Molecular knitting

MANY monomers, like styrene, can polymerize ionically. The incomplete polymer chain terminates in an electric charge; a monomer molecule reacts with it, the charge jumps to the new end, and the action is repeated. So the growing head of the chain carries a charge along with it.

Now a charge moving in a magnetic field travels in a circle. So, says Daedalus, a polymer chain extending in such a field will grow in a circle. The reaction should give that elusive rarity, a closed-loop ring polymer. Random brownian displacements may prevent perfect closure on the first circuit, but sooner or later the growing, winding snake will bite his own tail.

In a dilute solution, individual ring molecules will result. But in strong solutions or pure liquid monomer, the crowded molecular loops will inevitably grow through each other. The result will be a unique 'molecular chain mail' — a three-dimensional lattice of interwoven loops.

Molecular chain mail will have intriguing properties. Pulled in one direction it will shrink strongly in another, like a knitted pullover. It will not melt and will have a fantastic breaking strain, for it can fail only by the shearing of its chemical bonds. Made in a weak magnetic field, it will be soft and flexible, with large, floppy loop molecules; a strong field will give a tough solid with small, tight molecular links. It could even be made totally rigid by 'vulcanizing' it with a few cross links between the loops. Even so, it would not be brittle; for excessive loads would merely shear the cross links. The material would then 'work soften' locally, and yield slightly to relieve the concentration of stress.

More interesting still, molecular chain mail will have strongly nonlinear elasticity. It will yield readily to a small force, but as the molecular loops distort and pull taut, it will increasingly resist further extension. This is the subtle characteristic which gives biological tissues their remarkably high tenacity and freedom from catastrophic failure. It saves arteries, for example, from inflating locally under excessive pressure, and stops a bat's wing from tearing across if it catches on a thorn.

At last synthetic chemistry will be able to rival leather, catgut, sinew and other prized biological materials. Unbelievably pliable and strong, Daedalus's molecular chain-mail polymers will invade the market with a thousand uses, from shoes, handbags and coats to tubing, violin strings and balloon fabrics. And surgeons may welcome them as the ideal artificial material for soft-tissue repairs, fully compatible with the mechanical properties of their living surroundings. David Jones

Transmembrane protein of SIV

SIR—We would like to add to the observations on the transmembrane protein (TMP) of simian immunodeficiency virus (SIV) reported by Hirsch *et al.* in a previous scientific correspondence[1]. Immunoblot analysis of purified virus from a non-cloned pool of infectious SIV_{MAC}, isolated from a rhesus macaque infected with SIV_{MAC251} (virus supplied by R. Desrosiers) demonstrated the presence of a diffuse protein of relative molecular mass 42–46,000 (M_r 42–46K) (Fig. 1*a*). This virus pool, produced by co-cultivation of monkey peripheral-blood lymphocytes with phytohaemagglutinin-stimulated human cord-blood lymphocytes for 17 days followed by growth on the human C8166 T-cell line for 20 days, induces fatal AIDS-like disease in rhesus macaques[2] and is intended for use as a vaccine challenge stock. Further passage of this virus in C8166 cells resulted first in a population displaying proteins with M_r of both 42–46K and 31–37K (Fig. 1*c*) and later a predominance of 31–37K material (Fig. 1*d*). Nucleotide sequencing of polymerase chain reaction (PCR) amplified proviral DNA corresponding to the TMP-coding region of the SIV *env* gene revealed the presence of an in-frame stop codon in all six clones derived from the more highly passaged material, whereas none of the eight clones derived from low passage material contained a stop codon within this region (Fig. 2).

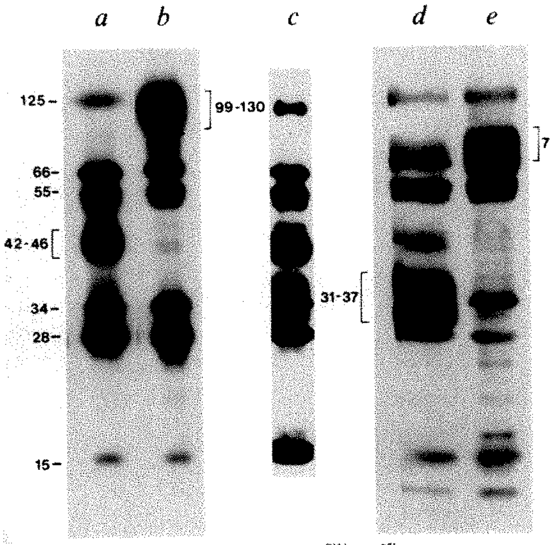

FIG. 1 Virus was purified by ultracentrifugation and chromatography on Sepharose 4b from cultures of infected C8166 cells. The cultures were passed every 3 or 4 days by feeding with uninfected cells in a ratio of 1:3. Tracks *a*, *b*, pass 6; track *c*, pass 14; tracks *d*, *e*, pass 18. Tracks *b*, *e* were applied in sample buffer without SDS. Virus antigen was electrophoresed on 12.5% polyacrylamide gels containing SDS and proteins detected by probing with serum from an SIV-infected monkey followed by ^{125}I-labelled Protein A. Relative molecular masses (K) were estimated from standards.

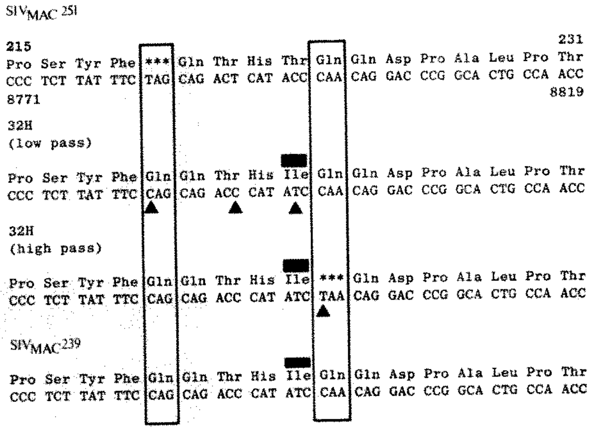
$SIV_{MAC}251$
215
231
Pro Ser Tyr Phe *** Gln Thr His Thr Gln Gln Asp Pro Ala Leu Pro Thr
CCC TCT TAT TTC TAG CAG ACT CAT ACC CAA CAG GAC CCG GCA CTG CCA ACC
8771
8819
32H
(low pass)
Pro Ser Tyr Phe Gln Gln Thr His Ile Gln Gln Asp Pro Ala Leu Pro Thr
CCC TCT TAT TTC CAG CAG ACC CAT ATC CAA CAG GAC CCG GCA CTG CCA ACC
32H
(high pass)
Pro Ser Tyr Phe Gln Gln Thr His Ile *** Gln Asp Pro Ala Leu Pro Thr
CCC TCT TAT TTC CAG CAG ACC CAT ATC TAA CAG GAC CCG GCA CTG CCA ACC
$SIV_{MAC}239$
Pro Ser Tyr Phe Gln Gln Thr His Ile Gln Gln Asp Pro Ala Leu Pro Thr
CCC TCT TAT TTC CAG CAG ACC CAT ATC CAA CAG GAC CCG GCA CTG CCA ACC

FIG. 2 Comparison of the DNA and amino-acid sequence of a portion of transmembrane region of the *env* gene of SIV_{MAC}. DNA was isolated from infected C8166 cells at low or high passage. PCR-amplified TMP-coding region was cloned and sequenced by standard procedures and the results compared to those published for two molecular clones of SIV (ref. 6). ▲, Nucleotide change; ■ amino-acid change, ★★★, termination codon.

We then analysed virus-derived antigen for the presence of oligomeric TMP. It has been reported for HIV-1, that heat stable oligomers of TMP may be detected by immunoblotting when SDS concentrations are reduced in the PAGE sample buffer[3]. Both full-length (Fig. 1*a*,*b*) and truncated (Fig. 1*d*,*e*) SIV TMP were found to form such oligomers; the estimated relative molecular masses indicate, in both cases, that these complexes are composed of dimers or trimers or both.

An experimental whole-inactivated-virus vaccine, currently under trial in rhesus and cynomologus macaques contains full-length and truncated TMP (Fig. 1*c*). Because infected animals exhibit antibody responses to peptides from the cytoplasmic C-terminal domain of SIV TMP (ref. 4) and this region modulates growth properties of the virus, at least *in vitro*[5], we believe inclusion of this region in putative vaccines to be important.

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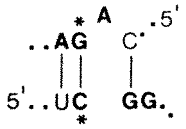
1. Hirsch, V. *et al. Nature* **341**, 573–574 (1989).
2. Cranage, M.P. *et al.* in *Proceedings of the International TNO Meeting on Animal Models in AIDS* (ed. M. Horzinek) (Elsevier/North Holland Biomedical, in the press).
3. Pinter, A. *et al. J. Virol.* **63**, 2674–2679 (1989).
4. Franchini, G. *et al. AIDS Res. hum. Retrovir.* **4**, 251–258 (1988).
5. Chakrabarti, L., Emerman, M., Tiollais, P. & Sonigo, P. *J. Virol.* **63**, 4395–4403 (1989).
6. Naidu, Y.M. *et al. J. Virol.* **62**, 4691–4696 (1988).

Genetic code origins

SIR—We wish to point out a remarkable circumstance associated with the recent characterization of the guanosine/arginine-binding site in *Tetrahymena* intronic RNA.

F. Michel and colleagues[1], elsewhere in this issue, show that nucleotide pair G264·C311 contributes to the specificity of this part of the catalytic site, and suggest that G264 is hydrogen-bonded to the guanosine/arginine ligands. The remarkable circumstance is that this sidechain- and stereo-selective arginine site[2] is formed by the conjunction of two arginine-encoding triplets, AGA and CGG:



The base pairs marked with vertical bars form the end of the conserved helix, conventionally called P7, and the arginine codons are in bold type. The critical base pair is between the asterisks.

Arginine has six possible codons, and arginine codons comprise 6.1 per cent of the contiguous triplets in the *Tetrahymena* intron. Given an arbitrary choice of two nucleotides, the probability that both lie within a codon for arginine (in any phase) is 0.029. The above observation would not, therefore, be sufficiently rare in itself to imply more than coincidence. In fact, the CGG codon itself is easily dismissed as coincidental. It is not phylogenetically conserved, instead being rather rare among the 66 tabulated group I intron sequences[3].

The AGA codon, however, is different. Although the P7 region is well conserved, there is considerable variation at the AGA codon. Among the 66 group I sequences there are 32 AGA codons (48 per cent), 31 CGA codons (47 per cent), and 3 AGG codons (5 per cent) occurring at the same positions in the sequence. However, these three sets of triplets all encode arginine. Thus, although there is variation on both sides of the completely conserved G264 residue, this variation produces triplets that represent both sets of modern arginine codons, AG^{A}_{G} and CGN.

All self-splicing group I RNA's so far examined have an arginine-binding site at this same location[4]. Sequence variations flanking the G residue that is apparently in contact with site-bound arginine produce three arginine codons and no others. The unbroken prevalence of this pattern in modern RNAs indicates that the sequences at this point must have also been arginine codons in ancient group I RNAs. This supports the suggestion[4] that this specific RNA–amino acid interaction may have been a progenitor of the genetic code. The code, on this basis, probably developed from an ancient RNA codon–amino-acid interaction, as somewhat originally suggested by Woese[5].

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1. Michel, F. *et al. Nature* **342**, 391–396 (1989).
2. Yarus, M. *Science* **240**, 1751–1758 (1988).
3. Cech, T.R. *Gene* **73**, 259–271 (1988).
4. Hicke, B.J. *et al. EMBO J* **8**, 3843–3851 (1989).
5. Woese, C.R. *et al. CSH Symp. quant. Biol.* **31**, 723 (1966).

Homoeopathic test

SIR—The practice of homoeopathy depends on the use of medicaments prepared in a special way. Solutions derived from a variety of parent substances are progressively diluted, with a dilution factor of 1 in 100 at each stage. The resulting fluid is said to acquire a specific 'potency'. In clinical use it is essential that the medicament chosen shall be appropriate to the patient's condition; fundamental to this is the contention that the various potencies can be distinguished from one another. Some years ago, I put that contention to the following test.

In consultation with an eminent homoeopathic practitioner, a former President of the Faculty of Homoeopathy, two specific potencies were selected for the test, namely, 'Natrium muriaticum 30C' and 'Sulphur 30C'. They are said to have strikingly different properties and to be strongly active. The practitioner declared himself quite confident that there could be no possibility of confusion between them.

A set of 20 uncontaminated bottles were numbered and then loaded with one or other of the medicaments, using a set of random numbers to decide which medicament should be placed in each bottle. Each bottle was enclosed in a metal can and the whole batch was sent to the practitioner without revealing the key.

No constraints were placed on the practitioner's methods, whether chemical, physical, clinical, parapsychological, or even magical, for identification of the potencies, as the question at issue was whether any distinctions could be detected regardless of means. But the practitioner kept to the routine he used in clinical practice for matching remedies to ailments. In all, he carried out 19 complete and reputedly independent tests on the same set of 20 bottles between April 1958 and May 1967, and reported his final conclusions in March 1970.

TABLE 1 Hypothetical and measured distributions from homoeopathic tests

	I	II	III
Calculated	$n = 19$	$n = 20$	$n = 380$
1. $p = 1.0$	19.0	20.0	380
2. $p = 0.9$	17.13 ± 1.3	18.0 ± 1.34	342 ± 5.85
3. $p = 0.7$	13.3 ± 2.0	14.0 ± 2.05	266 ± 8.93
4. $p = 0.6$	11.4 ± 2.13	12.0 ± 2.19	228 ± 9.54
5. $p = 0.5$	9.5 ± 2.18	10.0 ± 2.24	190 ± 9.22
Measured	10.63 ± 2.14	10.1 ± 2.55	202

TABLE 2 Results of comparisons using Student's t-test

	I	II	III
	$n = 19$	$n = 20$	$n = 380$
1. $p = 1.0$	reject	reject	reject
2. $p = 0.9$	reject	reject	reject
3. $p = 0.7$	$t = 3.9$ (reject)	reject	reject
4. $p = 0.6$	$t = 1.09$	$t = 2.49$ (reject)	$t = 2.72$ (reject)
5. $p = 0.5$	$t = 1.59$	$t = 0.13$	$t = 1.3$

The results were analysed in three ways: I, the number of correct identifications per set of 20 different bottles examined in a single run; II, the number of times each bottle was correctly identified in the 19 attempts on the bottle; III, the total number of correct identifications overall.

These scores are compared with those expected on the following hypotheses: 1, that the potencies are identifiable with certainty (that is, probability $p = 1$) as originally envisaged by the practitioner; 2, 3, and 4, that a correct identification could be expected in 90, 70, or 60 per cent of trials respectively ($p = 0.9$, 0.7 or 0.6, respectively); 5, that the observed number of correct identifications could have been arrived at by some random procedure which carries no information about the nature of the items ($p = 0.5$).

Table 1 sets out the means and standard deviations expected under each of these 5 hypotheses, for each of the 3 groupings of data, together with the actual values found. Table 2 sets out the conclusions from the various comparisons on the basis of Student's t-test at the 5% level.

The result for I ($n = 19$) cannot be distinguished either from the distribution for $p = 0.6$ or from that for $p = 0.5$ (chance). The other comparisons lead to the rejection of all the hypotheses that involve some discriminative power, leaving only hypothesis 5, indicating that the results might just as well have been arrived at by chance alone.

For a truly blind trial, each bottle should have contained material with a variety of possible activities or no activity at all. The practitioner may have been influenced by the expectation that each bottle should show activity of one or other type, in equal numbers and with no blanks, thus making the task of identification easier. Despite this bias, the statistical tests do not support the claim that the two potencies can be distinguished, at least by the methods used by this particular practitioner. It is possible that such a distinction could be derived from their clinical use, but practitioners are understandably reluctant to carry out a double-blind clinical trial that involves giving a human patient the wrong medicament. Perhaps a veterinary practitioner might be less reluctant.

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Amazon flooding

SIR—In his News and Views article on Amazonia during the ice-ages, P.A. Colinvaux referred to my hypothesis that the Amazon Basin was flooded to the 250–300-m contour by a great freshwater lake during the last glacial from about 45,000 BP (but not during previous glacials as might have been understood from the text). This lake, of course, was held in by a dam across the Amazon River, not by dams across its tributaries, which were also flooded, as Colinvaux stated. As well as draining north through the Orinoco, the lake would have drained south through the llanos of Bolivia to eventually reach into the South Atlantic.

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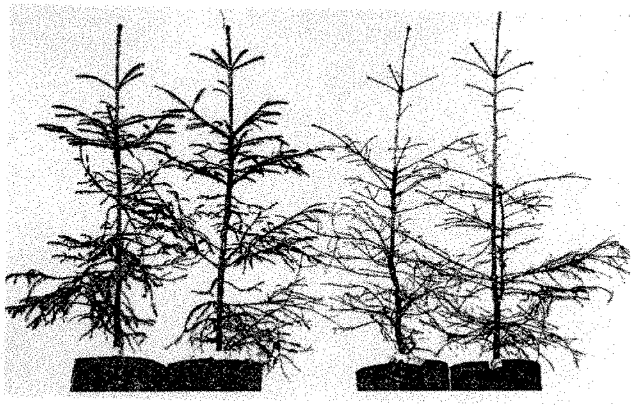
Springer books on Air Pollution and Forest Decline



D. Binkley, C. T. Driscoll, H. L. Allen, P. Schoeneberger, D. McAvoy

Acidic Deposition and Forest Soils

Context and Case Studies of the Southeastern United States

1989. VIII, 149 pp. 34 figs. (Ecological Studies, Volume 72). Hardcover DM 98,– ISBN 3-540-96889-X

Contents: Introduction. – The nature of soil acidity and H^+ budgets. – Magnitudes and patterns of nitrogen and sulfur deposition in the South. – Forest soils of the South. – Previous evaluations of sensitivity of southern soils. – Nutrient cycles and nutrient limitations in the South. – Simulation of the potential impacts of acidic deposition on forest soils in the South. – Synthesis and recommendations. – References. – Index.

E.-D. Schulze, University of Bayreuth; **O. L. Lange,** University of Würzburg, FRG; **R. Oren,** Duke University Durham, NC (Eds.)

Forest Decline and Air Pollution

A Study of Spruce (Picea abies) on Acidy Soils

1989. Approx. 495 pp. 186 figs. (Ecological Studies, Volume 77). Hardcover DM 298,– ISBN 3-540-50793-0

During the last decade, forest decline has become increasingly apparent. The decline in forest health was often reported to be associated with air pollution. The present study on Norway spruce stands in the Fichtelgebirge analyses various processes interacting within forest ecosystems. It covers transport and deposition of air pollutants, the direct effects of pollutants on above-ground plant parts, the responses of soil to acid rain, and the changing nutrient availability, and the accompanying effects on plant metabolism and growth. The role of fungi, microorganisms and soil animals in the decline of these stands is also assessed.

The volume is concluded with a synthesis evaluation of the influence of different factors, and their interactions on forest decline.

F. Scholz, Großhansdorf; **H.-R. Gregorius,** University of Göttingen, FRG; **D. Rudin,** Umeå, Sweden (Eds.)

Genetic Effects of Air Pollutants in Forest Tree Populations

Proceedings of the Joint Meeting of the IUFRO Working Parties
Genetic Aspects of Air Pollution
Population and Ecological Genetics
Biochemical Genetics
held in Großhansdorf, August 3–7, 1987

1989. VIII, 201 pp. 34 figs. Hardcover DM 75,–
ISBN 3-540-50858-9

Air pollutants provide environmental conditions that drastically differ in many respects from those to which forest trees are naturally adapted. Leading experts in the field here consider these questions of immediate relevance arising from the changing environment:

(1) Do air pollutants introduce effects of selection that diffe from those known for populations that are not subject to suc stress conditions?

(2) If air pollutants introduce quantitatively or even qualitatively novel selective effects, which consequences might arise from the adaptation of forest tree populations to the present conditions as well as for the preservation of adaptability to future conditions?

In addition to these questions, concepts for preservation of genetic resources are discussed.

In preparation

W. H. Smith, Yale University, New Haven, CT

Air Pollution and Forests

Interaction between Air Contaminants and Forest Ecosystems

2nd ed. 1989. Approx. 515 pp. 120 figs. (Springer Series on Environmental Management). Hardcover DM 198,–
ISBN 3-540-97084-3

This book is a comprehensive synthesis and overview of the complex interactions between atmospheric contaminants and forest ecosystems under low, intermediate, and high dose conditions. The author reviews the ability of forests to function as both a source of and a sink for air contaminants, and analyzes all interactions from an individual tree as well as a total ecosystem perspective. The general arrangement of the first edition has been retained, but the text has been expanded and thoroughly updated in recognition of the rapid progress of research in this field.

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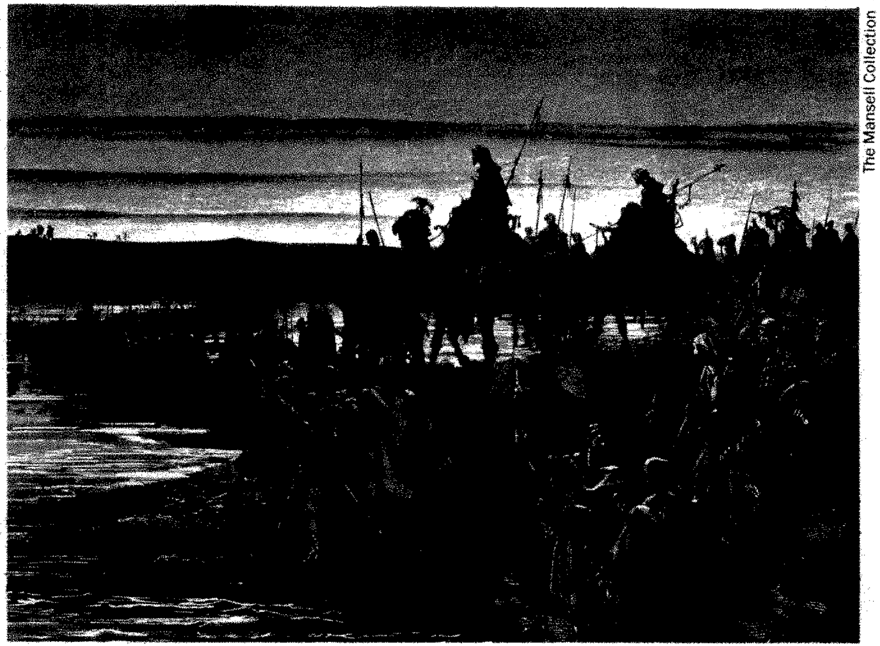
A turn in the weather

Peter J. Parr

Out of the Desert? Archaeology and the Exodus/Conquest Narratives. By William H. Stiebing, Jr. *Prometheus: 1989. Pp. 265. $21.95, £16.50.*

OLD Testament history used to be relatively simple. The Bible was, after all, generally recognized as the most complete and detailed — as well as the most readable — historical source to have come down to us from the pre-classical world, and one did not have to be a fundamentalist to believe, more or less, what it said.

But then, as a result of the literary criticism of the early part of this century, it became clear that the historical books of the Old Testament were far from being a unified narrative; rather, they were a mosaic of documents that had been written at various times, often centuries after the events they purported to describe, and had been edited with varying degrees of scholarship by a number of different people on a number of different occasions. The burgeoning archaeological discoveries in Palestine during the middle decades of the century did little to improve matters. After some initial optimism that the spade was beginning to uncover undoubted traces of the events of biblical history, it emerged that archaeology was as unsatisfactory and tantalizing a guide as the written word. The result is that for almost every aspect of the history of ancient Israel, there is now a wide range of often totally contradictory hypotheses available. All of them are capable of explaining at least some of the supposed facts; but none is convincing enough to command universal, or even majority, respect.



On the go — the children of Israel crossing the River Jordan.

The Mansell Collection

Of nothing is this more true than of the events that lie at the heart of Israel's history, and are the foundation of its religion and its national identity: its occupation of the land of Canaan and its dispossession of the previous inhabitants (the Exodus and Conquest). A vast literature has grown around the problems of chronology and causation surrounding these events. Most scholars have their own individual theories, but, as Professor Stiebing shows in the excellent volume under review, there are four or five basic hypotheses.

The first, and longest established, is the most conservative (with the exception of the real fundamentalist approach), and accepts the fact of an Israelite exodus from Egypt followed by an essentially forceful subjugation of Canaan, though not necessarily in all the particulars given in the biblical account. The second is of almost equally long ancestry. It also bases itself fairly closely on the biblical text, but sees the settlement of the Israelites more as the result of peaceful infiltration into the less densely inhabited areas of Canaan by semi-nomadic pastoralist groups from the desert fringes. A third model, proposed in the early 1960s, has proved popular with historians adopting the fashionable socioeconomic stance. It postulates an entirely internal development within Canaan, a sort of 'peasants revolt' against oppression by the ruling classes and the flight of disaffected groups away from the urban centres and towards more remote parts of the land, where their relative isolation and sense of common grievance and purpose fostered their eventual growth into a nation.

Finally, there are what Stiebing calls composite or hybrid theories which have developed from the other models. One of these sees the original Israelites not as social and political revolutionaries, but as refugees escaping from the onslaught of more warlike neighbours, such as the Philistines; another accepts the semi-nomadic pastoralist origin of the Israelites, but argues that they were not newcomers from outside the country but had been in Canaan for generations, living in symbiosis with the town-dwellers and becoming sedentary only when the Canaanite urban centres on which they depended collapsed. Just when any of these processes or events took place is also a matter of controversy; current suggestions range between the twenty-third century BC to the eleventh, though most serious scholars accept the thirteenth as the most likely, whichever theory is adopted.

By far the greater part of this book is devoted to a discussion of the arguments for the various theories and dates, each of which has its strengths and weaknesses. The task has been done many times before, but rarely as lucidly, succinctly and judiciously as by Professor Stiebing. After an admirably straightforward account of the principles of biblical literary criticism and a review of the relevant Palestinian archaeological material, the evidence for and against each hypothesis and each suggested chronology is presented fully and fairly, and is critically assessed. None of the theories is found to be wholly satisfactory, and it is this somewhat negative and disappointing state of affairs that leads Professor Stiebing to an eminently sensible conclusion, namely that "one problem with all of the models we have considered is that they view the Israelite conquest or settlement in relative isolation". As he points out, the final centuries of the second millennium in the eastern Mediterranean and western Asia saw some of the most dramatic and turbulent events of ancient history: the collapse of the Hittite, Egyptian and Mycenaean empires, and the appearance of the Dorian Greeks, the Sea Peoples and the Aramaeans, as well as of the Israelites.

Were all these events connected; did they have a common cause? Stiebing thinks they were, and argues that it was widespread drought resulting from climatic change which led to the economic, social and political disturbances throughout the region. The argument is not new, and the entire subject of the influence of climatic change on the course of human history is one which has for long engendered heated debate between determinist and anti-determinist schools of historical interpretation. The evidence from the climatological and geological sources which Stiebing reviews — though certainly less fully and critically then he does that from archaeology and the texts — is not as conclusive as he implies, if only because there is still far too little of it. But the argument remains attractive and intellectually satisfying, even if intuitive, and helps establish an 'ultimate cause' of the Exodus and Conquest.

The final question, however — which Stiebing does not address — is whether such an 'ultimate cause' really matters. A distinguished historian, Geoffrey Elton, once said that his colleagues tended to spend too much of their time on causes and not enough on results. Climatic change is a phenomenon of concern to the scientist, but what should interest the historian, and what he should seek to explain, are the different reactions of different people to the same challenge. The disturbances at the end of the second millennium may well have their origin in the same climatic events, but the consequences in various parts of the Mediterranean, Western Asia and Egypt were very different. In Canaan they resulted in the creation of the Israelite nation; and the fact that the Israelites believed their nation to have been created by Exodus and Conquest is of much greater interest and importance than whether it was really true. ☐

Peter J. Parr is in the Department of Western Asiatic Archaeology, Institute of Archaeology, 31-34 Gordon Square, London WC1H OPY, UK.

More heat for the debate

R. S. Pease

Nuclear Fusion. By Keishiro Niu. *Cambridge University Press: 1989. Pp.240. £35, $59.50.*

JAPAN'S research into controlled nuclear fusion became established as a substantial national programme in the 1970s, and involves a remarkable partnership between the universities, the Japanese Atomic Energy Research Institute and engineering industries. About a dozen universities have research programmes; there is a central institute devoted to plasma physics and magnetic confinement at Nagoya, and a leading institute for lasers and inertial confinement at Osaka.

Professor Niu is head of the theory group of the department of energy studies at the Tokyo Institute of Technology, and has contributed to research in fusion reactor studies. His book, which is a graduate-level text for engineering science, was originally published in Japanese in 1979 with M. Suguira of the Electrotechnology Laboratory, Tsukuba, as co-author. Now it has been translated and partially updated by Professor Niu, who presents the principles of controlled thermonuclear fusion in high-temperature plasmas confined by magnetic fields and by inertia.

The merit of the book is the wide range of topics covered. The introductory chapter leads to the Lawson criterion for the degree of confinement required for a net energy output from thermonuclear reactions, given in a form suitable for quasi-continuous systems. A chapter on magnetic confinement covers the basic properties of charged-particle motion in magnetic fields, the concept of magnetic pressure and the various heating methods. Most space is given to the Tokamak — currently the leading magnetic confinement system — and to instabilities which restrict the configurations that can be used, and those which may restrict the thermal insulation. Other magnetic systems are dealt with briefly.

The chapter on inertial confinement describes the basis of the compression and heating of small spheres of deuterium-tritium fuel by focusing onto them powerful beams of pulsed laser radiation and of charged particles. Here the author elaborates on the problems of the absorption of the radiation, describes the lasers and the methods of generating and focusing particle beams. The final chapter, on reactor design, describes some of the engineering issues, touches on resources of materials and safety, and describes three of the reactor concepts that have been developed: for the Tokamak, for mirror confinement systems and for inertial confinement, respectively.

The book deals hardly at all with experimental results, and it is disappointing to have no account of the impressive Japanese achievements in that area. Professor Niu has given himself insufficient space to expound some of the topics adequately — reading the book, one feels rather like a member of a touring party led by a ruthless guide, determined to show us everything, however imperfect our appreciation. He has also been poorly served by the proof-readers. The equation of motion describing the balance of electromagnetic forces with plasma pressure gradients is corrupted by misprints, for example; the reverse-field pinch configuration is said to arise from a diamagnetic effect, whereas it is paramagnetic; and *aficianados* of mistranslation will appreciate the "Miller" field for magnetic confinement described in Chapter 2, but translated correctly as the "mirror" only in the last chapter. Taken altogether, the book is a useful but not wholly accurate guide to the principles of hot fusion. ☐

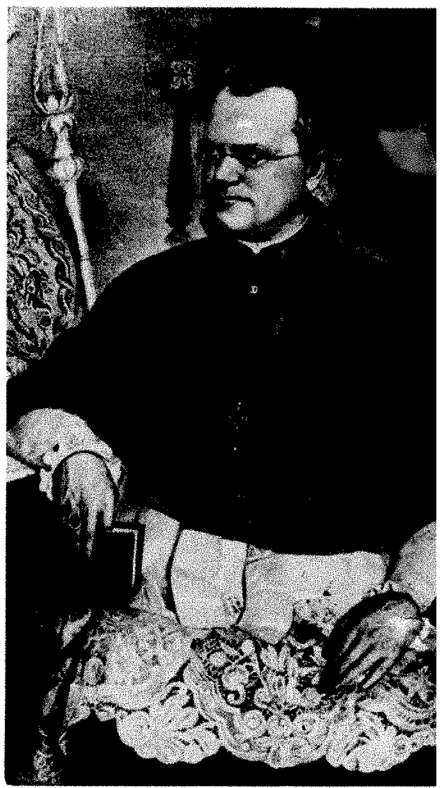
R. S. Pease was formerly Director of Fusion Research at the UK Atomic Energy Authority.

Peas before swine

J. S. Jones

The Mendelian Revolution: The Emergence of Hereditarian Concepts in Modern Science and Society. By Peter J. Bowler. *Athlone, London: 1989. Pp. 207. £35, $29.95.*

THE sociology of science bears the same relation to research as pornography does to sex: it is cheaper, easier and — as it is constrained only by the imagination — can be a lot more fun. This book discusses the changes in our thinking about heredity and evolution over the past 200 years. Those of us who teach genetics like to present the subject as unfolding logically from Mendel, through Morgan, to Crick and the latest heart-throb of molecular biology. We all accept that there may be some self-delusion here, but it makes us feel good about the rigour of our own field, and it keeps at least a few of the students awake.



Mendel's work was thought 'irrelevant'.

Bowler sees this as a conspiracy by geneticists to hide the subject's past. In this slim volume (and at a price which will make its purchasers expect something Rather Special) he argues that scientific progress makes sense only in its historical context, and that this is particularly true of the study of inheritance because of its use in justifying social policy. As he says, "most historians have little interest in defending the old view that science offers completely objective knowledge". In pursuit of this thesis, Bowler produces some interesting insights into early genetics. Few of us now realize just how unimportant the mechanism of inheritance appeared to nineteenth-century biologists. They were much more interested in cells, in development and in the nature of species, and what we now call genetics was seen as a minor part of each of these fields. Mendel's work was not ignored because it was not understood; rather it was thought to be irrelevant. Modern genetics could not begin until the study of the transmission of biological information was separated from that of its translation, and this book contains a great deal that will change the views of geneticists about the beginnings of their subject.

It is less satisfactory on the evolution of genetics in the twentieth century. Many would quarrel with the claim that Morgan became interested in the chromosome theory because it gave him access to funds, and that in the 1950s to study cytoplasmic inheritance was to side with communism. And is it really true that Kammerer's work on the inheritance of acquired characters was never duplicated only because he had an unrivalled ability to breed midwife toads in captivity? Bowler ends by pointing out the irony that molecular biology has now turned the study of inheritance full circle by casting doubt on the concept of the gene as a unit.

The book sees genetics primarily as the raw material of history and philosophy. It is a study of science and society and, of its kind, a good one. No doubt in a hundred years we will have similar treatments of selfish DNA as an example of Thatcherite greed, or of genetic imprinting and the women's movement; and there will arise new Poppers, Kants and Kuhns — a sort of philosophical Joy of Sects — all anxious to give biologists the instruction manual of how Science is Actually Done. On the tomb of a philosopher buried just across the path from Herbert Spencer (who makes an unexpected appearance in these pages as a social lamarckist) is the epitaph "The philosophers have only interpreted the world in various ways. The point, however, is to change it". Alter the object of the first sentence to 'science' and most geneticists will agree with Marx's interpretation of their intellectual priorities. ☐

J.S. Jones is in the Department of Genetics and Biometry, University College London, 4 Stephenson Way, London NW1 2HE, UK.

- *The Extended Phenotype*, by Richard Dawkins, has recently been reissued in paperback by Oxford University Press. The book is the sequel to *The Selfish Gene*, and was reviewed in *Nature* **296**, 506 (1982). Price £6.95.
- *Toward a New Philosophy of Biology* by Ernst Mayr is also now out in paperback. Published by Harvard University Press $14.95, £11.95. See review in *Nature* **333**, 25 (1988).

Drawing back the cloak

Ralph A. Lewin

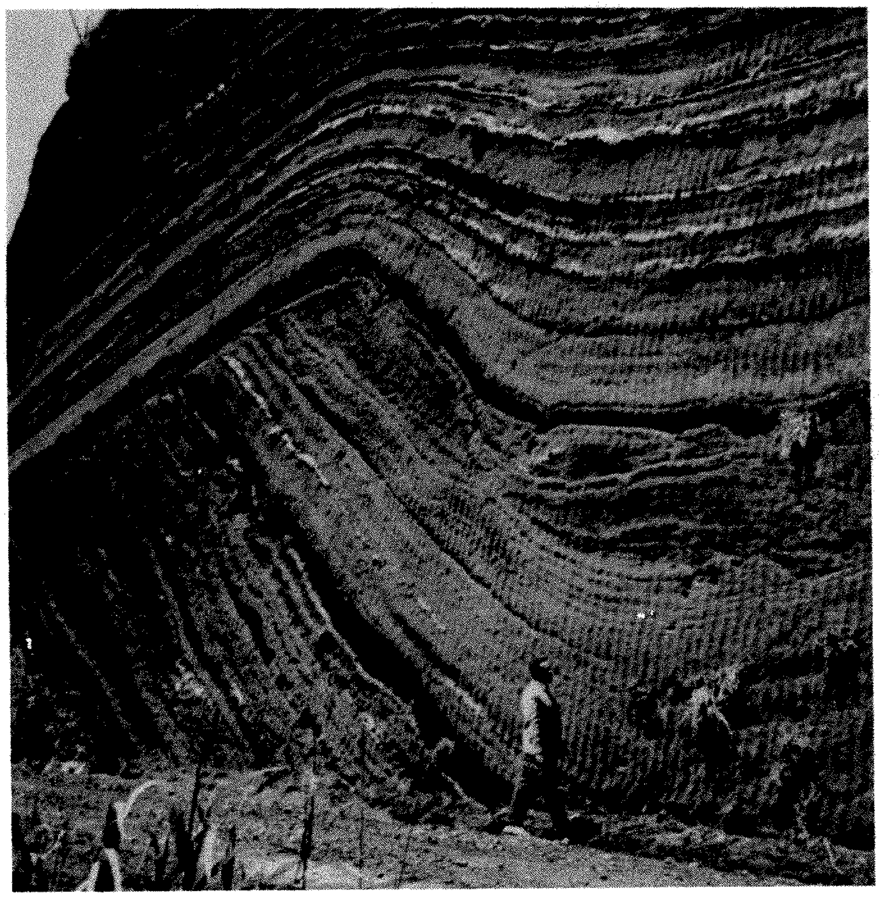
The *Chlamydomonas* Sourcebook: A Comprehensive Guide to Biology and Laboratory Use. By Elizabeth H. Harris. *Academic: 1989. Pp. 780. $145, £90.*

THE name *Chlamydomonas* — from the Greek, 'the cloaked one' — suggests something melodramatic, mysterious, perhaps even furtive. But when Ehrenberg introduced the name in 1833 he could have had no idea how much melodrama would emerge from the study of this simple-looking, single-celled green alga, or how much mystery would remain even today.

The genus is a large one, comprising many hundreds of species: a few marine, more in freshwater ponds and streams, and lots more in soils, even in deserts, from polar regions to the tropics. Put a teaspoonful of soil into a glass of water, add a pinch of fertilizer, set the glass in a window, and in a couple of weeks you'll assuredly have a culture with one or more kinds of *Chlamydomonas*, their microscopic cells swimming by means of paired anterior flagella.

To identify them further a reference work will be needed. So far, the best available has been a detailed taxonomic compilation in German, by H. Ettl (*Nova Hedwigia Beih.* **49**, 1–1122; 1976). Now there is an equally scholarly and valuable book to tell us almost everything else that we need to know about the genus — or, rather, to outline what has been discovered, how and by whom, what still needs to be studied, and how we should set about it. The scope of *The* Chlamydomonas *Sourcebook* is vast, but Libby Harris has been equal to the task. We have here a paragon of excellence.

The book presents, in clear, readable English, an overview of the huge and (almost certainly) polyphyletic genus, with chapters on cell structure, motility (those two simple-looking flagella have proven to be fantastically complicated machines), photosynthesis and other metabolic processes, and genetics of nuclear and non-nuclear genes (chiefly those of the chloroplast, now probably the best known in all biology). There are also detailed instructions for the cultivation, mutagenesis, mutant selection and conservation of strains along with some very useful



Volcanic ash layers on a road cut in Oshima Volcano, Japan. Airfall material, from volcanic eruption, forms distinct layers that follow the slope of the ground. Two generations of ash fall are seen in the picture, the beds are not folded; the inclined layers reflect the original attitude of ash accumulation. The photograph is reproduced from *Volcanoes*, by R. Decker and B. Decker, a revised and updated edition published by W. H. Freeman. Price is £11.95, $14.95.

information on the ones in current use. (As in the Bible, there is a 'begat' chapter with all sorts of details of family histories, carefully researched by Harris before memories fade and sources are forever forgotten.) Also included are descriptions of many useful biochemical techniques and methodologies for molecular biological work on such unicellular algae.

There are, too, good reviews of the sexuality of these versatile microbes, the feature which, above all else, led to their selection for detailed studies. Seventy years ago, the first direct confirmation of mendelian inheritance came from a study of *Chlamydomonas*, but for 30 years after that there were few publications on this subject (and many of those, regrettably, proved to be largely fictional). Only in the late 1940s did a handful of biologists, seeking a single-celled plantlet with genetic potential, take up studies of the genus. Mostly we chose to work with one of two unrelated species, *C. moewusii* (also known as *C. eugametos*) and *C. reinhardtii*. Their sexual proclivities are sometimes melodramatic, or even furtive, but by now they have been elucidated fairly well and are under good laboratory control. Unfortunately *C. moewusii*, which lends itself best to studies of sexuality, suffers from a metabolic disability all too common among algae, an inability to grow heterotrophically in darkness, so in this species photosynthesis cannot be bypassed. Consequently *C. reinhardtii*, with its more versatile metabolic potentials, has been the species of choice.

If I have to find a bone to pick, it would be on this point. When you read in Harris's book something about *Chlamydomonas*, you can generally assume that it relates to *C. reinhardtii* unless otherwise specified. However, generalizations are dangerous in a 'genus' as diverse as this — what's true for Peter may not be applicable to Paul — and doubtless examination of other species will reveal exceptions and surprises of all sorts.

I note that on p.175 the first equation, misquoted from Chiang *et al.*, is incorrect, and that on p.130 *C. philotes* is wrongly given as heterothallic. But I have discovered nothing of importance that's missing, and lots of fascinating details that I knew nothing about before. There are full references to almost 3,000 publications, and abundant tables, diagrams, photographs and other illustrations, with adequate scales. A good ten-page, three-column index is provided. It's all there, in a stout binding and a hard, waterproof cover. The book will inevitably need both. ☐

Ralph A. Lewin is in the Marine Biology Research Division, Scripps Institution of Oceanography, University of California San Diego, La Jolla, California 92093, USA.

Far off and exceeding deep

Harry Shipman and Noel Hart

Deep Time: The Journey of a Single Subatomic Particle from the Moment of Creation to the Death of the Universe and Beyond. By David Darling. *Bantam, London/Delacorte, New York: 1989. Pp.192. £12.95, $17.95.*

From Quarks to the Cosmos: Tools of Discovery. By Leon M. Lederman and David N. Schramm. *W.H. Freeman: 1989. Pp.242. £16.95, $32.95.*

HOW did the Universe begin? Where will it end? These questions have taken on a new, rich dimension in the 1980s with the development of a connection between cosmology and particle physics. In producing these two books, Darling and Lederman and Schramm join the crowd of scientists and journalists writing on the subject for a 'popular' audience (whatever that is).

Deep Time is a 'prose poem' telling the story of a proton which journeys from the Big Bang to the end of cosmic evolution. Some episodes in this journey illustrate a different and entertaining way of describing astronomical phenomena. Our proton hero narrowly escapes being sucked into a black hole in the centre of a galaxy, and along the way there is a nice description of the current central dogma about quasar energy sources.

But any writer takes on a real challenge in writing a book-length 'poem'. Lewis Carroll pulled it off in *Alice in Wonderland*, but Darling overdoses the reader with phrases such as "the hot breath of genesis", "oases of order" and "the mighty fortress of the second law" (of thermodynamics). One of us (H. S.) found the language merely bothersome; the other (N. H.) found it virtually unreadable.

It is hard to draw distinctions between well-founded theory and scientific speculation in a poem. Darling doesn't bother. For instance, he implies that cosmic strings must be midwives that assist in the birth of galaxies. Yet we don't even know whether cosmic strings exist. Fiction comes in, too, with tales of Prospero, the tenth planet in the Solar System. What tenth planet? Inexpert readers are here being led into the land of the tabloids, where the difference between science, pseudoscience and science fiction is considered to be irrelevant. Such readers will also have to struggle with sentences that presume a more than passing familiarity with, for instance, antileptons and antibaryons.

Lederman and Schramm provide a more conventional treatment of cosmology and its interface with particle physics. The pictures are outstanding and serve well to illustrate the sparse text, while Lederman's perspective as an experimental particle physicist shows through nicely. Many pages clearly and thoroughly, though ponderously, describe mammoth particle accelerators and the associated array of detectors. Most popular books on this topic let the stork deliver new particles, such as the omega-minuses from the clear blue sky. The authors here give us the facts of life, describing the 50,000 pictures which had to be taken before the omega-minus appeared to confirm the symmetric view of nature based on the quark model.

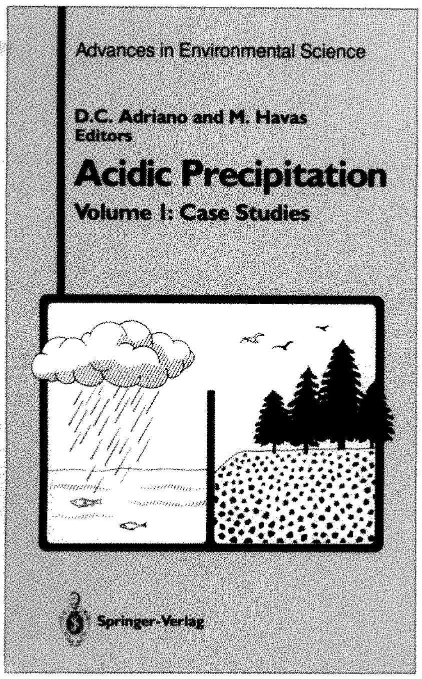
Lederman and Schramm, however, use jargon injudiciously. The book's second sentence describes the control room of Fermilab's Tevatron, but those who don't know what or where the Tevatron is must wait until page 115 for a pleasant, four-page description of it. And the beat goes on: in the first five pages the reader is sprayed with bullets of terminology: antiprotons, p-bars, accumulator storage rings, luminosity, deflection 'kicker' magnets, GeV, CDF, standard model quarks, leptons and more. One-sentence definitions of some of these bits of scientific lingo are not enough for the nonspecialist. Several pages later the level plunges precipitously. Using a box, the authors patiently explain powers of ten, and they take ten pages of text and many gorgeous illustrations to show that atoms are made of electrons and protons. Both reviewers, one of us a scientist and one a non-scientist, found the text to be like a rollercoaster ride and just as disorientating.

There is a plethora of cosmology books on the market already, including, of course, Hawking's blockbuster *A Brief History of Time*. Why write or publish so many of them? That's a question the book-buying public should be asking.

Harry Shipman is in the Department of Physics and Astronomy, and Noel Hart the Department of Foreign Languages and Literatures, University of Delaware, Newark, Delaware 19716, USA.

- An English translation of Paolo Maffei's *L'Universo nel tempo* is now available; *The Universe in Time*, published by MIT Press, addresses issues not covered in Maffei's first book, *Beyond the Moon. The Universe in Time* is structured as a voyage in time, looking at the life cycle of the stars, planetary systems and eventually geological and ecological evolution. Price $29.95, £26.95.
- Also recently translated, from the French, is Jean Heidmann's *Cosmic Odyssey*. The book was written to "give a description of the fabulous richness of the cosmos". It explores the relativistic universe, the quantum universe and the inflationary universe. *Cosmic Odyssey* is published by Cambridge University Press, price £11.95, $19.95.

Featuring an innovative 5-volume subseries on acidic precipitation



Advances in Environmental Science

D.C. Adriano and M. Havas
Editors

Acidic Precipitation

Volume I: Case Studies

Springer-Verlag

D. C. Adriano, University of Georgia, Aiken, SC; **W. Salomons**, Haren, The Netherlands (Eds.)

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Subseries

Acidic Precipitation

D. C. Adriano, University of Georgia, Aiken, SC; **M. Havas,** University of Toronto, Ont. (Eds.)

Volume 1:

Case Studies

1989. XIV, 311 pp. 113 figs. Hardcover DM 228,– ISBN 3-540-96929-2

Contents: *D. W. Johnson, S. E. Lindberg:* Acidic Deposition on Walker Branch Watershed. – *E. Matzner:* Acidic Precipitation: Case Study Solling. – *A. H. Johnson, T. G. Siccama, W. L. Silver, J. J. Battles:* Decline of Red Spruce in High-Elevation Forests of New York and New England. – *C. S. Cronan, R. A. Goldstein:* ALBIOS: A Comparison of Aluminium Biogeochemistry in Forested Watersheds Exposed to Acidic Deposition. – *G. Abrahamsen, H. M. Seip, A. Semb:* Long-Term Acid Precipitation Studies in Norway. – *J. T. Turk, N. E. Spahr:* Chemistry of Rocky Mountain Lakes. – *N. van Breemen, P. M. A. Boderie, H. W. G. Booltink:* Influence of Airborne Ammonium Sulfate on Soils of an Oak Woodland Ecosystem in the Netherlands: Seasonal Dynamics of Solute Fluxes. – *F. T. Last:* Acidic Deposition Case Study Scotland. – *M. Hauhs:* Lange Bramke: An Ecosystem Study of a Forested Catchment. – Index.

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Brazil walks the tightrope

Brazilian researchers survive with admirable calm amid an economic tempest. With strong nerves and good luck, the first democratically elected president in decades may next year bring better times.

The first round of elections to choose Brazil's president has presented the country with its first true choice since the military takeover in 1964. In last week's free and democratic vote, two candidates with diametrically opposite views were chosen to go forward to the final vote on 17 December. Both candidates are young but their backgrounds and outlooks are utterly different.

Fernando Collor de Mello (40) is wealthy, handsome and conservative. His main, perhaps only, claim to fame is the action he took to cut the salaries of 'maharajas' — enormously overpaid government officials — while governor of the small north-eastern state of Alagoas. His National Renovation Party was set up simply to promote him and has no ideological or class base. Facing him is Luíz Inácio da Silva, known simply as 'Lula', a former lathe operator and leader of the autoworkers' union. Almost exactly ten years ago he led a strike of three million workers that opened the first cracks in military rule.

His Workers Party is well-organized, well-established and supports a redistribution of income, regulation of capitalist enterprise and a moratorium on the repayment of the foreign debt.

So far there has been no sign that the military will intervene in the electoral process unless forced to do so by a major social upheaval. But if Lula is elected next month, the temptation may be strong, and there will be anxiety in Brazil. One factor staying the military's hand will be the knowledge that they have no idea how to tackle Brazil's colossal economic problems.

The best the winner can hope for when he takes over from President José Sarney in January is that the economic condition of Brazil will be no worse than today. Brazil is close to terminal hyperinflation and economic disintegration. In the five years during which Sarney has been president, nothing has been done to tackle the underlying problems.

Last year, inflation came close to 1,000 per cent. Now it is running twice as fast, at over 30 per cent a month. In daily life, the evidence of inflation is immediate and tangible. Take the 100,000 cruzeiro note; in 1986 it was worth almost ten dollars, the biggest note in common use. Three years later, the note comes as change for the smallest of purchases: it is worth less than one cent.

Money is not something solid in Brazil, it is constantly slipping away and vanishing. Fixed salaries are halving in value in less than two months; unpaid bills a few months old are not worth collecting, and items ordered a few months ago will double or triple in price before they arrive. Brazilians never know how much anything costs — only what it used to cost. Restaurants do not print prices on their menus but just leave spaces to be filled in anew each day. Taxi drivers have their meters set at some historical rate and then consult an official table – which always appears freshly issued — to convert the reading to today's charges.

Inflation has left a bewildering trail of banknotes. The 'cruzado' was created in 1986 in the Cruzado plan, the first of the government's two big attempts to halt inflation. It is worth 1,000 of the old cruzeiros. The next attack on inflation, the Summer plan, created the 'new cruzado' worth 1,000 of the old cruzado. Notes of all three currencies are in circulation, meaning that the face value of some has to be divided by a thousand and others by a million.

Dealing with inflation obsesses everyone. Salaries are constantly being revised and re-revised to try to keep up, Congress is always tied up with supplementary budgets to restore some value to budgets granted earlier. Anyone with any significant sum of money rushes it — immediately — into high-interest accounts. It is possible to make almost two per cent a day, 55 per cent a month. Industry can deal in special inflation-indexed Treasury bonds and keep itself afloat. But for those on fixed incomes, and for the poor, it is a different matter.

With thanks

One person and one person alone made possible the four-week trip to Brazil upon which this survey is based. Ricardo Bonalume Neto, a science journalist at the newspaper *Folha de São Paulo* and occasional contributor to the news pages of *Nature*, kindly volunteered to set up appointments for me within Brazil, a task which is made triply difficult by frequent breakdowns in Brazil's long-distance telephone system. Despite the problems, Ricardo always succeeded in reaching the unreachable and making appointments with the totally unavailable — and always with just moments to spare. In São Paulo, special thanks go to Ricardo for driving me around town in his vintage Beetle, despite a series of punctures.

Alun Anderson

Brazil contains vast inequalities. In the wealthy area behind Copacabana beach, every apartment building has its guard. Late at night they are the only people on the streets. Not far away one million people live in *favelas*, shanty towns — they too are visible from the beach. In São Paulo, shacks of wood and cardboard are crammed under elevated motorway intersections and line the abandoned land along the city's polluted rivers. Altogether 25 million Brazilians live in slums out of a population of 130 million.

The statistics of inequality are staggering. The top five per cent of the population control almost 50 per cent of the nation's wealth, the next 25 per cent take 30 per cent; the remaining 70 per cent of the population share 20 per cent. More than 20 million people do not have an adequate diet, five million children have never attended school, 20 per cent of all adults are completely illiterate and fewer than half can read a newspaper.

But then comes the great contradiction. Despite its third-world distribution of wealth, Brazil does not have a third-world economy. Brazil is the world's eighth largest economic power, with a GNP of $250,000 million and a $20,000 million trade surplus — the world's largest after Japan and West Germany — on exports of $33,000 million. And those exports are not the agricultural products and raw materials of a third-world nation; 70 per cent of them are manufactured products including aeroplanes, automobiles, industrial machinery, tanks, and even missiles.

Thanks to the power of the economy, the top quarter of the population — 30 million or so people — are middle class with much the same tastes in clothes, food and culture as their European or American counterparts. In the book shops in São Paulo, the bestsellers are the same as in New York — Umberto Eco, Milan Kundera and even Stephen Hawking's *Uma breve historia do tempo*. Look one way and Brazil is part of the developed world — look the other way and it is part of the third world. Brazil is in transition. What of the future? Is Brazil about to make the leap to developed-nation status or fall back to third-world status? ►

If Brazil is to emerge from its difficulties it will have to tackle its huge deficit. It is doubly hampered; it owes a huge amount of money — over $100,000 million — to foreign bankers and it has a colossal internal debt. The oil money borrowed during the 1970s was poured into vast state-owned enterprises: oil, steel, chemicals, electricity, railways and telecommunications. State capitalism may have kick-started Brazil's development but it never proved much good at providing profits. Today, the state enterprises are a huge drain on government resources. They have never been left to follow market realities but are used for a variety of political purposes. When convenient, electricity prices are set to manipulate the rate of inflation or jobs are given out to relieve unemployment in a sensitive area.

The need to finance the debt of the state enterprises is one of the factors that triggered inflation. As foreign loans vanished, the government turned to the internal market or printed money. And as the government is the guarantor of huge quantities of money in index-linked savings and bonds, it had to borrow to pay for inflation, driving the inflation rate yet higher.

The state enterprises are a social as well as an economic phenomenon. They created a new breed of Brazilian — the technocrats. Well-paid and enormously powerful, they will prove reluctant to work anywhere else. So will the army of workers they control. For all but the richest, Brazilian life is permeated with a fear of falling — falling into the abject poverty below. In a state sector job security is total — and wages are higher. Every year more jobs are created and labyrinthine bureaucracy grows.

It will be a brave politician who tackles the state enterprises. Sarney made one brief sally in January when he announced plans to dismiss 60,000 state employees (one result would have been that almost half the staff at the research facilities described below would have been out of a job). But he backed down in the face of public opposition, saying the matter was the responsibilty of Congress.

What will Collor or Lula do? Collor has so far been careful to cultivate his image while not committing himself to any definite policy, although he has said he is in favour of the sale of state enterprises. Lula's views are clearer. Corruption and nepotism are to go from the state enterprises but the enterprises themselves will remain and perhaps even be strengthened.

Without tackling the deficit it is difficult to see how the Brazilian economy can recover. But there are other things that can be done to help, Bolivia providing one example. In 1985, Bolivia's inflation rate reached 23,000 per cent but now it is under 10 per cent. Hyperinflation was ended by getting rid of the deficit, in part by the unorthodox method of reducing tax rates to the level where people — particularly those operating in the black economy — would pay them rather than cheat. Tax revenues rose several thousand times as a result. In Brazil, some economists believe that 50 per cent of the economy may be black. If that is the case, then changing the tax structure to bring them into the legitimate economy, and removing excess regulation that makes it hard for them to expand, could provide the boost Brazil needs.

Despite the sense of impending economic catastrophe, the visitor is surprised to find that Brazilians themselves are rarely downcast. Amid all the difficulties, it would be hard to find a Brazilian who does not believe the nation is destined for greatness. They may joke that "Brazil is the country of the next century — and always will be", but underneath there is unfailing optimism. They know that the ecomony can be dynamic, they know that the country is vast and vastly rich with minerals, oil and gas. When pressed, that confidence slips into the kind of xenophobia which inspires politicians and military men to threaten to cancel the foreign debt and to tell the rest of the world to go to hell.

It is in this spirit that Brazil's politicians talk about the Amazon — its their great frontier, the American West, that will be conquered and bring untold riches. The development of the Amazon is inevitable; the only questions are when and how.

But international pressure has put the environment on the political and election agenda. Some tax subsidies for 'development' of the Amazon that have encouraged giant cattle ranches have already gone, and if the pressure is kept up, others may go too. But politicians know that the main force driving poor settlers to Rondonia is poverty; expansion in the industrial heartlands would be the best hope for the Amazon, buying time until better development strategies have been worked out. But to say that is simply to return to the problem of inflation.

In the pages that follow, it will be readily apparent that scientists have no escape from the economic, political and social realities that surround them. Scientists worry about inflation, financial instability (which sends some institutes close to bankruptcy), sudden reversals in policy and the wave of democracy which is altering old power structures within research institutes. It is a time of transition. Among the cast of characters that appear in the remainder of this survey are the giants of the older generation, people who weathered the period of military rule or who had to flee into exile, a new breed of research administrators who know the routes through the labyrinths of Brasilia, frustrated young researchers fresh from foreign research laboratories, and those who have put aside personal goals to train the next generation of researchers and work for development. And of course there are some bureaucrats and technocrats too.

People will be found to disagree on many things, as may be expected. The reader must also be warned that not everything told to *Nature* is necessarily true. In Brazil, charm is a national art, and even scientists may sometimes be unwilling to let facts interfere with a well-told tale. Charm is necessary. Amid Brazil's inequalities it makes life bearable, even enjoyable. At one end of the spectrum, charm blends into the kind of good-natured, smiling persistence that is often necessary to get anything done in Brazil. Further along it turns into the skill of creating imaginative compromises and ways around rules (the '*jeito*' of which Brazilians are so proud). Yet further along it turns into corruption plain and simple.

In the opinion of this reporter, one category of researchers is special. Those who live and work in the Amazon are set apart by the extraordinary paucity of their resources and by the unique interest of their research into one of the world's least understood ecosystems. Brazil could buy some very inexpensive international prestige by deciding to make research in the Amazon a high priority — after all, the Amazon (or the greater part of it) is Brazil's alone. That research could include not only the full range of biological and physical sciences but also linguistics. With 170 separate Indian languages in Brazil, there is a unique opportunity to take the world lead in the field. This has been partially recognized in a special government project on Indian languages, but the level of activity is still far too low.

There are several other simple things the government could do to help researchers. One would be to remove completely the bureaucratic obstacles that slow the import of scientific equipment and reagents. Government officials say that rules must be applied, but that is not what is in dispute — it is appropriate rules that are needed, not those that regard all imported items as the same.

Other problems are not so easy to deal with because they are financial (the level of funding, the freeze on new appointments in the universities) or political (the unwillingness of the government to allow excellence to be rewarded and incompetence punished among the university's tenured staff).

Clearly the research milieu is now changing and there will be increasing emphasis on interaction with industry, and with boosting the dismally low level of research and development within industry. A few researchers are already seeing the future and making their own plans for new links, others might be wise to imitate them. □

ORGANIZATION

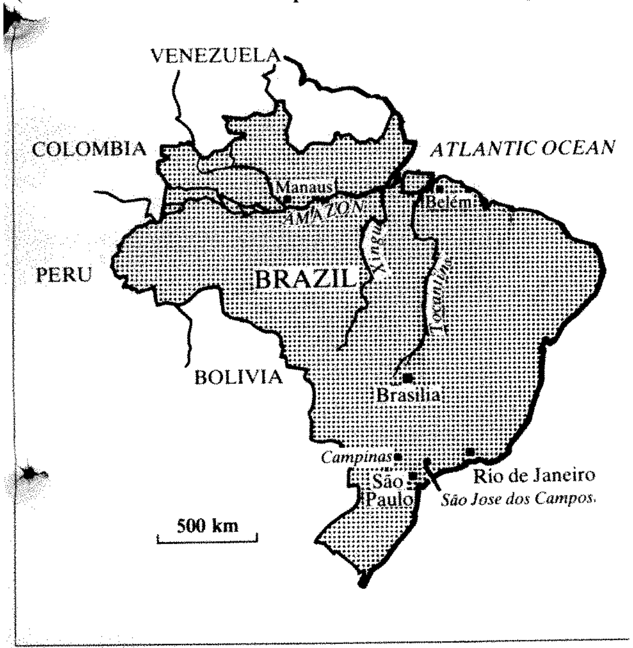
Whistle-stop guided tour

THIS page provides a map, both geographic and institutional, of science in Brazil. As with any map, its principal appeal is to people who dislike travelling without always knowing exactly where they are. Those who are content simply to arrive may turn to the following pages.

Universities. Brazil has more than a million students enrolled at its federal, state and private universities, but there are only eight universities carrying out active research in a wide range of scientific disciplines.

In a category of its own, well ahead of any competitor, is the University of São Paulo (page 368). It produces more than half the doctorates in Brazil. Second comes the University of Campinas (UNICAMP), located 100 kilometres north of São Paulo (page 368). USP and UNICAMP are not supported by the federal government but by São Paulo state which is Brazil's richest, generating 50 per cent of total gross national product (GNP).

Third is the best of the federal universities, the Federal University of Rio de Janeiro (page 358). After Rio, the rankings are open to dispute (and, of course, any university may have particular specialities in which it excels). But most agree that in the next five places will be the Pontifical Catholic University of Rio de Janeiro (remarkable as the only private university with strength in science, page 359), the State University of São Paulo (yet another of São Paulo's state-funded universities, with campuses throughout the state), the Federal University of San Carlos (again located in São Paulo state), the Federal University of Rio Grande do Sul (located in Porte Allegre in Brazil's southernmost state), the University of Brasília, and the Federal University of Minas Gerais (located in the state capital of Belo Horizonte).



Research institutes. At one time or another, all of the major research institutes belonged to what everyone still knows as the National Research Council and refers to as "CNPq". (The council's change of name to the National Council for Scientific and Technological Development has proved unpopular, page 373). Eleven research institutes still belong to CNPq; for historical reasons they are concentrated in Rio de Janeiro. Two major research institutes, for space research and amazonian research, are now attached directly to the what most people refer to as the Ministry of Science and Technology. In fact, the name is not correct. In a dramatic political battle, the ministry was absorbed by another ministry early this year but fought its way out again, victorious and independent, as the Special Secretariat for Science and Technology of the President of the Republic (page 371) a ministry except in name. The research council, CNPq, is answerable to this body.

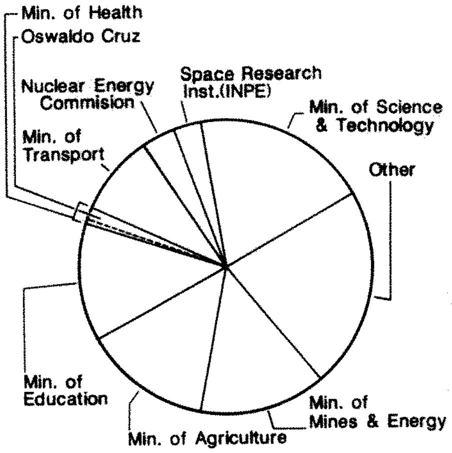
There are many other research institutes — for Meteorology, Metrology, Mining and so on — attached to other ministries but they are less central to *Nature*'s interests. Exceptions are the Oswaldo Cruz Foundation of the Ministry of Health, and the EMBRAPA chain of research institutes attached to the Ministry of Agriculture (pages 370 and 363).

Research support. Virtually all support for scientific research comes from the government. The total budget in 1987, the last year for which complete figures are available, came to $1,640 million, approximately 0.7 per cent of GNP. The following year the budget rose but in January 1989 massive cuts were ordered by the President. As supplementary budgets are still being debated by Congress it is not possible to say accurately how much will be spent this year, although the final figure could be as low as half that granted in 1987. In that year the budget was divided up among ministries as shown in the pie-chart below, with the Ministry of Science and Technology receiving $470 million (35 per cent of the total). Of that $470 million, just over half ($249 million) went to CNPq to support its scholarships, research grants and institutions (page 373). Thirty per cent went to the Agency for Financing Funds and Projects (FINEP), which is part grant agency and part commercial bank (page 372), and to two other ministry funds, the National Fund for Scientific and Technological Development (FNDCT) and the Programme of Support for Scientific and Technological Development (PADCT). The latter is particularly important because it is through this fund that the World Bank has made a $72 million matching loan to Brazil to help in the development of the science base (page 372).

Major research institutes

CNPq
Rio de Janeiro
  National Observatory (p.359)
  Museum of Astronomy and Related Sciences
  National Laboratory for Astrophysics (p.374)
  Brazilian Center for Research in Physics (p.361)
  Institute of Pure and Applied Mathematics
  National Laboratory of Scientific Computing
  Center for Mineral Technology
Belém
  Emílio Goeldi Museum (p.363)
Campinas (SP)
National Synchrotron Light Laboratory (p.368)
Brasília
  Brazilian Institute for Scientific and Technical Information
  Centre of Science and Technology Policy Studies
Special Secretariat (Ministry of Science and Technology)
São Jose dos Campos (SP)
  National Space Research Institute (p.367)
Manaus
  National Institute for Amazonian Research (p.362)
Ministry of Health
Rio de Janeiro
  Oswaldo Cruz Foundation (p.370).

Outside of the Ministry for Science and Technology, support for basic research comes from the Ministry of Education's Coordinating Agency for Advanced Training of High Level Personnel (CAPES), which supports graduate training in the universities (page 372). Individual states also have their own funding agencies, São Paulo's FAPESP is the best known and biggest (page 372). □



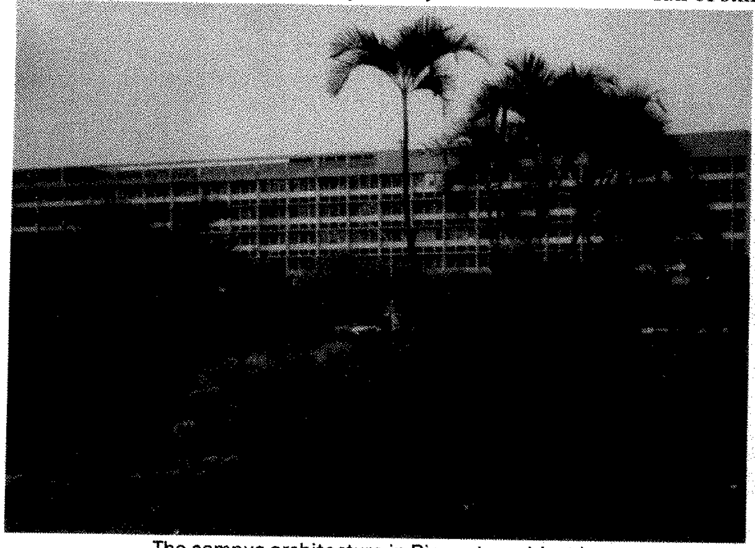
The distribution of government research spending by ministry during 1987.

Island exile for academics

Rio de Janeiro

FOR all its carnival image, Rio on a rainy day is about as exciting as Manchester, and considerably uglier. Rio is more than a few famous beaches and a soccer team; it is a city of 5.7 million people (a million of them living in slums), an industrial centre and a major port. Heading north out of Rio, the Guanabara Bay elevated expressway runs for two grimy miles alongside rows of wharves, cranes and warehouses before winding out of the city through the industrial Zona Norte. There are no tourists out there.

But Rio in the sun is another city altogether — certainly one of the most beautiful in the world — not because of anything man has built but because of its mountains and the sea. The famous Sugarloaf is remarkable only for its symmetry among an infinite variety of mountain forms — some bare rock and some covered with lush forest — spilling down to the sea and a series of beaches, bays and lagoons. There is nothing to add to the cliched speechlessness of Charles Darwin who wrote, visiting on the *Beagle* in 1832, that the landscape "so completely surpasses in magnficence all that the European has ever beheld in his own country, that he knows not how to express his feelings".



The campus architecture in Rio: not an object lesson.

Rio residents add utility to aesthetics; they like to say that the only reason that major riots do not break out in the slums — the *favelas* — is because of the open beaches and overwhelming beauty of the surroundings. While the idea of beauty as a palliative for poverty may seem romantic, in Rio it is believable.

Rio was long Brazil's capital and tries to claim the honour of being the first city to open a proper university, as against faculties or schools for the study of individual disciplines. But the motive for setting up the university was not entirely scholarly, or so the story goes. Diplomatic necessity was the driving force. The King of Belgium was about to pay a state visit to Rio, and the president found that he had no university to confer a suitable honorary degree upon his distinguished visitor. The University of Brazil was thus created, although it was not to last for long. Some of that university's buildings still remain in an old part of town, with a pleasant view across the bay to the Sugarloaf. Alongside it are two of the research institutes of the National Research Council (CNPq): the Brazilian Institute of Physics (CBPF) and the National Laboratory of Scientific Computing (LNCC). A third CNPq institute — the Institute of Pure and Applied Mathematics — also occupies a delightful site overlooking the botanical gardens on the lagoon behind Ipanema. And not far from there, also surrounded by greenery and mountains is the Catholic University, PUC.

But Rio's main university, the Federal University of Rio de Janeiro, enjoys no such picturesque setting. Almost the entire university was moved in 1972 to a low, uninhabited island, five miles from downtown Rio, just north of the docks of Guanabara Bay. Many academics claim that the the university was moved for political reasons, so that out on the island the military could be sure the students could foment no trouble.

Other academics claim the story is an exaggeration; they say the island is simply a manifestation of the "gigantism" that comes easily to the totalitarian mind. The island is artificial, created by filling in the land between eight tiny islands, and took almost twenty years of intermittent work to complete. It is two miles long and for the most part, covered in an untidy scrub. At one end stands a vast university hospital, at the other a cluster of university faculties, housed in concrete blocks, but each so far from its neighbour that it seems unlikely that there are many spontaneous meetings between the members of different faculties. Rows of students wait at the bus stops of each faculty or try to hitch a ride back to Rio. There is nowhere to live nearby.

Optimists say that it is too early to judge the project as the island will gradually fill in — new research institutes linked to private and state companies are already being built — and become an academic city. But it seems unlikely that the island will really begin to feel lived in and friendly before the end of the century. The university has 25,000 students and 3,000 faculty. Overall, it is usually described as the third-best university in Brazil, but its biophysics group is the best and its reputation in biochemistry and engineering, where the university runs a very large and innovative graduate school (COPPE), is very high.

The university faces the now classical problem of other federal universities — growth has stopped. For the past four years a federal freeze has prevented the university hiring any full-time staff (there are ways of hiring people on specific contracts). People returning from post doctoral experience abroad must thus face the dismal prospect that the universities are full of still-young people, much less qualified than themselves, who joined the university a decade or more ago. It will be the next century before the hiring bulge of the 1970s fully works itself out of the university system.

Rio has always had the pick of good graduates so the problems are not so severe here, but Professor Luiz Pinguelli Rosa, director of COPPE, explains that many universities are "dominated by people who are not concerned with research, the old-fashioned teacher, professional and bureaucratic people who just come to class and then go home, and now with political appointees who have nothing to do. But now is a time of transition". He also points out a certain irony in the present situation. Many academics opposed the military and, in the late 1960s, large numbers were dismissed or even driven into exile. But despite the military's pressure on academic freedom, they expanded the universities and put money into science and technology. Now the government has lost the will to invest for the long term.

Despite the shortages it is possible to keep good groups going. Nelson Maculan, a COPPE professor, has a team of young researchers with postdoctoral experience at universities that read like a listing of the world's best. By early October he had already received 25 visiting professors since the start of the year. But his field is theoretical — operational research — and he needs only a work station and outside contacts to stay at an international level. He despairs of how to bring back good people who have gone abroad for training when he has no jobs to offer them.

Rio is also swept up in the wave of democracy passing through the universities, another reaction to the disappearance of the military. Everywhere students, and particularly support staff who greatly outnumber faculty, are asking for a bigger say in running the universities and in electing their rectors.

Rio has gone further than most, prompting the Minister for Science and Technology, Décio Leal de Zagottis, to claim that the a "crisis" is imminent that will harm the university for a decade, slowing its attempt to form new links with industry. The courts will ultimately have to decide whether, in de Zagottis's words, a "university can be directed by students, workers and professors, all at the same level". But academics at the university say the problems are being exaggerated, and Bio-Rio, a key biotechnology venture on the island (see page 374) says its immediate prospects are good. ☐

CATHOLIC UNIVERSITY

The art of living on the edge

Rio de Janeiro

ONE of the most studied announcements on the noticeboards at the Pontifical Catholic University of Rio de Janeiro (or PUC as everyone calls it) gives the latest prediction of when staff salaries might be paid. PUC is the only private university in Brazil carrying out a substantial scientific research programme, but this remarkable achievement is rewarded irregularly in these difficult times — the salaries for January, for example, did not arrive until April. "It is a very unstable situation", says Professor Humberto Brandi, Dean of the Technical-Scientific Center (CTC) which coordinates the university's scientific programmes. "We have constant problems of money . . . I don't think you can imagine how it works. It's completely crazy."

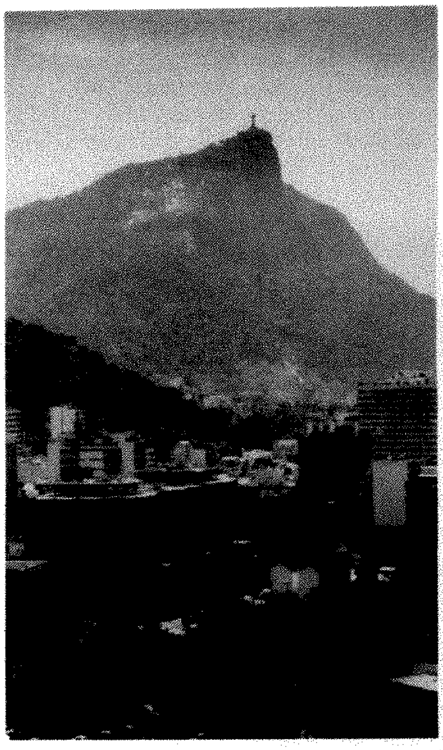
The private status of PUC makes it particularly vulnerable to economic instability as it does not have the guaranteed income of federal and state universities and must rely instead on student fees, and research grants and contracts.

PUC was set up in 1941 as Brazil's first private university. In 1959, the university bought Brazil's first computer, a gigantic US-manufactured machine that took up two floors and was part mechanical and part electronic. When the university found the computer could not be switched off, they were stimulated to appoint the first full-time staff. The university is still very strong in computer science and telecommunications; IBM is just about to donate a large mainframe computer and the telecommunications research centre (CETUC) receives large contracts from the state telecommunications organization, TELEBRAS.

The university has 8,000 undergraduate students, 3,000 of them in the Scientific-Technical Centre. The emphasis is on technology, rather than basic science. There are 1,000 graduate students in the centre, along with 260 faculty.

The faculty's funds and salaries come mostly from the research council FINEP (see page 372) and increasingly from research contracts (PUC has around 160 of them) from state and private industry. But early in October, Brandi is still waiting for September's salaries to arrive. In April, when they had been without salaries from January, he had been forced to borrow some money intended for investment in projects to pay part of the salary bill, then later repay the money, with interest. As interest rates are currently 1.8–2.0 per cent a day, this is a painful transaction. Asked why he appears so calm about it, he relies, "We have been living on the edge for ten years, we know how to do it".

This time, anyway, he says he knows the money for salaries is there, it just has to be "liberated". "Liberation" is a word that senior Brazilian researchers use often. It means a kind of guerilla-style harassment of goverment agencies until they deliver the funds that are owed. The budget is decided by the Ministry of Planning and is approved by Congress but the funds are disbursed by the Ministry of Finance. There the officials are under constant pressure from the International Monetary Fund to improve the deficit. One trick is to slow the pace of payment; because the budget is not inflation indexed, every delay means a decline in the value of the amount to be given out. Brandi and other "liberators" must thus spend their time on the telephone, and flying up to Brasília, trying to make the funds move down the pipeline.



The famous statue of Christ looks down on the Catholic University.

"Brazil is a young country and everything is very informal", says Brandi. "It's possible to pick up the telephone and talk to the minister. The ministry has its own priorities which are complicated, and works on pressure." Last month's salary has, at any rate, been liberated from the Ministry of Finance and travelled as far as FINEP. Brandi is now occupied with liberating it from there. ☐

NATIONAL OBSERVATORY

Counting pennies not stars

Rio de Janeiro

IT is the middle of October and Ramiro Muniz, vice-director of the National Observatory remarks, without any particular emotion, that the observatory is absolutely broke. "Today we do not have a single cent", he says. All that is left are some funds earmarked to pay the electricity bill.

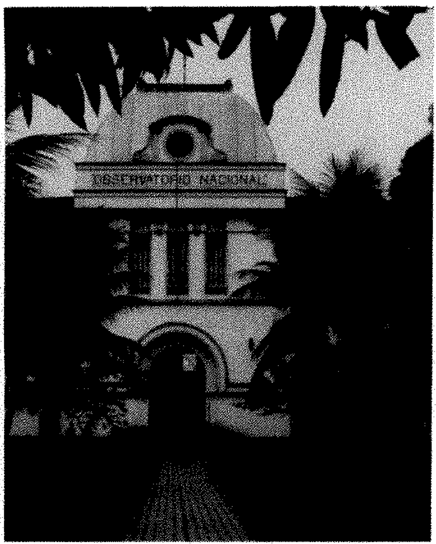
Muniz hopes that Congress will soon pass a supplementary budget, designed to resurrect funds that melted way under 30 per cent a month inflation. He explains that the observatory operating budget (which at the beginning of the year would have been worth $800,000) exists in a computerized account in Brasília. It bears no interest, nor is it corrected for inflation. It appears at the beginning of the year, and immediately begins to lose one per cent or so in value every day. The observatory tries to buy as much as it can immediately — but as Muniz says, "you cannot go hog-wild buying everything in January".

Anyway, the law prevents advance payment of government funds to private companies without special bank guarantees; it's payment on delivery, and by the time a delivery arrives, costs may have doubled, or tripled. . . .

Do-it-yourself becomes the only way to save money. The observatory has taken to mending its own cars and using its technical expertise to repair building — the workshop apparently produced a fine set of aluminium doors. Muniz clearly likes improvising. He is a physicist (and spent five years at Berkeley in the fifties) but most enjoys the times when he can use his knowledge of paramagnetic resonance in unconventional settings: verifying a colleague's hypothesis that a particular drug acts on trypanosomes through the release of free radicals, and finding that the magnetic signature left in rock cores can be used to recover temperature history. Now, necessity has made him an expert on economics and accounting. He expects no quick solutions to Brazil's problems. "The politicians are as lost as a blind man in a shoot out", he says, "they have no idea which way to run".

The observatory also has internal problems which are essentially a reflection of its origins. The observatory began in the early nineteenth century as a practical place: it made astronomical observations of use in navigation, recorded magnetic data and corrected compasses, and kept the hour, supplying a time signal at noon for ships in Rio de Janeiro harbour to set their chronometers.

The observatory (and its old telescopes) now stands on a little mango-tree covered hill in the São Cristovão region of Rio; its original hill, which was closer to the sea, was quarried away sixty years ago and used to extend Rio's waterfront. Today, the observatory is unhappily split amongst the descendants of its original functions. There are astronomers (who now see themselves as basic researchers), geophysicists (who have forged links with industry), and engineers who still keep the hour (and are looked down on by the first two groups). Muniz tries to prevent argument over the groups' respective merits, and impishly quotes Lewis Carroll on the reality behind words, " 'The question is', said Humpty-Dumpty, 'which is to be master — that's all' ". Muniz's view is that "it is less important who is in charge inside than for the observatory to be good enough to get funds from outside".



The National Observatory's roots lie in the early 1800s.

Brazil has maybe 100 working astronomers (the astronomy society has 300 members), the great majority of them either at the National Observatory or at the University of São Paulo. The main observing facility is the National Astrophysics Laboratory, associated with the National Observatory and located at Brasópolis in the mountains some 300 km from Rio. Groups from several universities use the facility, which is equipped with a 1·6 m reflector and 60 cm refractors. Brazil is not the best place for optical astronomy; there is too much cloud and not enough high mountain.

If support is forthcoming it is likely that Brazil will use a facility in the Chilean Andes, probably in collaboration with a US university group. Some observations are already made in Chile and Argentina, as well as in South Africa. Projects at the observatory include the South American part of the Harvard–Smithsonian redshift survey, a stellar astrophysics group that studies the polarized emissions from stars, and a wholly Brazilian project on T-Tauri, young stars.

Elsewhere in Brazil there is a 30-metre radiotelescope (now at INPE, at one time it belonged to the observatory) but it is already old. A consortium from the University of São Paulo and INPE and some other groups — the first time a consortium approach has been tried out in Brazil — is now attempting to find funds for a much larger radio array.

The observatory's geophysicists are responsible for Brazil's magnetic and gravimetric surveys. They also do seismic work with Petrobras, the state oil company. North-east Brazil is seismically active although no shock bigger than Richter 5 has been recorded. Much larger earthquakes are believed to have passed unrecorded in the Amazon, and there are plans to set up a seismic station at Manaus — when and if the money becomes available. □

SBPC

Science as continuous struggle

Rio de Janeiro

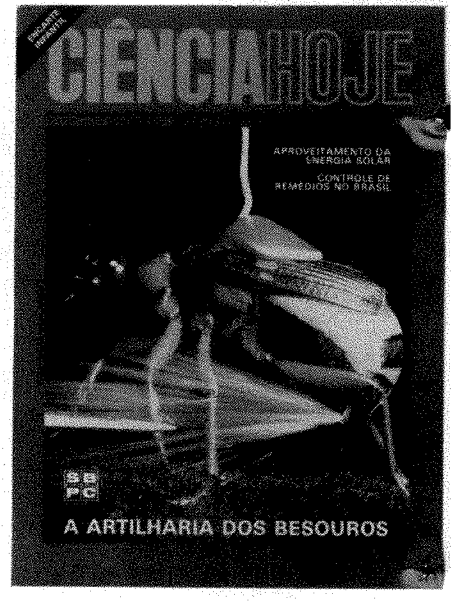
Ennio Candotti is a name known to virtually all Brazilian scientists, which is some measure of the continuing importance of the Brazilian Society for the Progress of Science (SBPC), of which he became president this year. SBPC is the exact equivalent of the AAAS in the United States, it organizes seminars and holds an annual meeting (which as many as 10,000 people attend), it lobbies the government on behalf of scientists, and publishes a scientific magazine.

Candotti is a busy man, constantly in demand. He is not without his critics, who fear he might not be big enough to force the government to provide science with the support it needs, or suspect he has become too much of a journalist after having spent seven years as editor of SBPC's monthly magazine, *Ciência Hoje*.

By training, Candotti is a theoretical physicist. Over a lunch, taken late in the kitchen of the Oswaldo Cruz Institute (the official lunch in the dining room had ended an hour earlier and all the guests had gone home, but a series of interruptions, and people queuing to talk to him, had so far prevented Candotti from actually eating anything), he characterized, somewhat melancholically, the state of Brazilian science as "continuous struggle".

"For each hour of investigation, there are ten hours of fight to get the money, to get equipment, to solve the problems of stability for your laboratory. Every person is divided between two jobs, one is the old job we used to have, the other is the fight to maintain the old job. . . . We dream of a stable budget, to have equipment, to have libraries, but we don't get them". The result is that Brazilian scientists have become very ingenious. "If you gave them half of what an average US scientist has, they would be able to achieve three or four times as much", Candotti says, half-seriously.

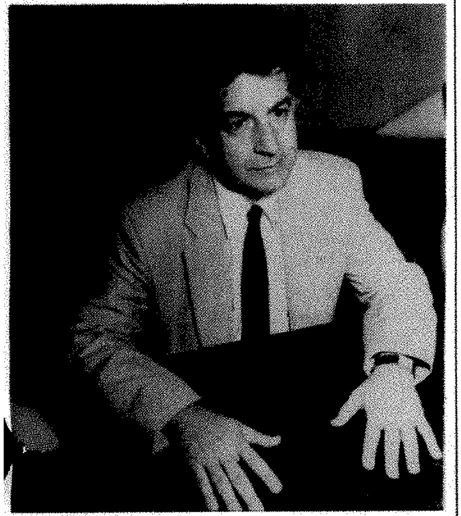
"Political interests are always present in scientific life," says Candotti. "Why? Because the whole Brazilian problem is political. Fifty million people are undereducated and without health care, 30 million are without basic instruction, 60 million earn less than $60 a month. At the same time we have a very powerful industry and economic growth. It is these contradictions that must be solved to give stability. We need 100,000 researchers, we have 30,000, we don't have people to do the most simple things. But the government does not have the stability itself to provide continuity to a programme for


ENCARTE INFANTIL
CIÊNCIA HOJE
APROVEITAMENTO DA ENERGIA SOLAR
CONTROLE DE REMÉDIOS NO BRASIL
SBPC
A ARTILHARIA DOS BESOUROS

SPBC's monthly magazine, *Ciência Hoje* (Science Today), has a circulation of 70,000 and is "written by scientists, rewritten by journalists", according to SPBC director, Ennio Candotti. It is modelled on *Scientific American* and *La Recherche*.

four or five years . . . 30 per cent a month inflation destroys all plans and budgets."

Inflation is something Candotti had direct experience with as editor of *Ciência Hoje*. "I buy so many tonnes of paper, but one month to the next costs can grow by 50 per cent; and by the time you receive the magazine subscriptions they are worth nothing, so how can you do the next issue?" Candotti's job now is part lobbyist for the scientific community. SPBC is not the only lobby group; the 60 scientific societies of Brazil also have a nine-person commission to lobby Congress and the ministries, and other groups represent individual universities and research institutes. Although some argue that the appearance of these new groups means that the SBPC is not as strong as it was, a better explanation might be that society is maturing and SPBC no longer need be the only opposition group, now that military ruler has ended.


SBPC's Candotti: The table is bare.

Candotti welcomes the appearance of other groups. "In the last year or so, society is becoming more organized, although there are no true political parties, there are more pressure groups. In the recent discussion of the new constitution, many groups were organized. Perhaps this will change the next government, it will not be completely free to govern by authoritarian methods, but will have to work with society. Congress has a new and important role".

So far, he says, there has been too much arbitrary political influence over scientific programmes. "If the minister changes, then the whole scientific enterprise can change 180 degrees. Elsewhere they are more pragmatic, here things can change from one direction to the opposite . . . all programmes, all priorities can be changed in a few months."

While Brazil may be more unstable, the job of lobbying the government is little different from elsewhere. Politicians give you attention "according to your political force, who you represent and who you can mobilize", says Candotti. ☐

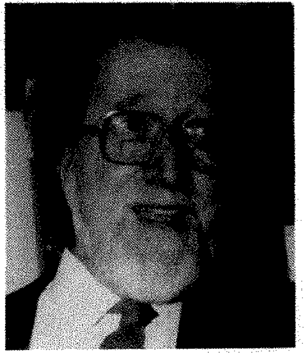
PHYSICS CENTRE

Physics in times good and bad

Rio de Janeiro

PROFESSOR José Leite Lopes' office in the Brazilian Center for Research in Physics (Centro Brasileiro de Pesquisas Físicas, CBPF), where he is director, is decorated with photographs of Wolfgang Pauli (who he knew when he was in Princeton), and Albert Einstein (taken on a chance encounter); the corridors nearby bear giant pictures of Richard Feynman and Brazilian colleagues, taken on one of his several stays at CBPF. The treasure among them is one of Feynman dressed as Mephistophiles for the 1952 Rio carnival.

Those early days were exciting — the beginning of physics in Brazil. In 1947 Cesar M. G. Lattes, then just 21 years old, had taken a set of exposed photographic plates from Chacaltaya, the cosmic ray observatory high in the Bolivian Andes, to C. F. Powell's laboratory in Bristol, England. The result was the discovery of the pions and their decay which was published by Lattes and Powell in *Nature* in 1947 and won Powell the 1950 Nobel prize. Leite Lopes, who was then in his mid-twenties, published his own work on meson decay and the theory of nuclear forces, also in *Nature*, in 1948. The next year, these two young scientists (Lattes, fresh from Berkeley, where he had won further fame and had appeared on the cover of *Life* magazine) set up the institute where Leite Lopes is now director. "The university", says Leite Lopes, "did not understand research, so we set up up our own organization, beginning as a private institute and helped by the prestige of Lattes". A few months later Feynmann came for a couple of months, he was back in 1951 for his sabbatical year, and again afterwards. There were other famous visitors too — J. Robert Oppenheimer, Eugene Wigner and Chen Ning Yang among them. But this lively period came to an end with the military coup in 1964. After being briefly detained, Leite Lopes moved to Paris, but returned to Rio in 1967. Two years later he was expelled by the military government. "I was clearly a progressive," he says. He moved first to the United States and then to the University of Strasbourg, where he remained in exile for 15 years, "working hard and living well — a gift from the generals". In 1985, with military rule ending, he was invited back, to be director of CBPF for the second time. In the meantime the institute had become one of the 11 CNPq research institutes.


Leite Lopes: returned exile.

Leite Lopes' "progressive" leanings still show; although the problems of the institute (simply put: "we have no money . . . if they take anything more we can only go to the beach") are foremost in his mind, he bunches his fists with anger when he talks about the state of Brazilian society, the ten million children living in *favelas*, and the breakdown of the public education system that had provided for poor people.

Back at the institute, Leite Lopes' main task has been to try to build the experimental physics that is largely lacking from the Brazilian scene, except at the University of São Paulo. The institute's own linear accelerators are 30 years old and no longer work. One success was the designation of the institute as the National Laboratory of Nuclear Fusion. But the plan, to study muon-catalysed fusion, exists only on paper.

Despite the lack of sophisticated equipment, several groups at CBPF look after themselves very well. Some have worked out ways to work with scientists abroad, others have adapted their science to the equipment available in Brazil. The institute has 93 researchers and more than a hundred graduate students.

The institute still has several projects going on at the Chacaltaya cosmic-ray observatory, now in cooperation with the University of Tokyo's Institute for Cosmic Ray Research. But the jewel in the crown is the group of 20 high-energy physicists, led by Joao Anjos, which works closely with Fermilab in the United States; the group even had President José Sarney pay Fermilab an offical visit in September. The group has built (at a surprisingly low cost) a parallel multiprocessor, a twin of the ACP machine at Fermilab, enabling all data gathered at Fermilab to be analysed back in Rio.

A group studying hyperfine interactions has taken the option of tailoring its science to available equipment, rather than gathering data abroad. CBPF pioneered research on the Mössbauer effect in Brazil, and has carried out the most complete Mössbauer studies of the new high-temperature superconducting materials.

Leite Lopes is thinking of returning to France to an emeritus professorship at Strasbourg and leaving the problems of Brazil to someone younger. His critics (of which he has rather few) do not mention his age, but that he is too big a figure for today's Brazil. The same comment is heard of others from his period (including University of São Paulo rector, José Goldemberg), particularly of those who spent time in exile. As one researcher put it, "only giants could still do good science through all those times". ☐

Fighting for the Amazon

Belém

ON the waterfront in Belém, flat-bottomed river boats, hammocks swinging between their open decks, bring all the curiosities of the Amazon to market. There are huge fish and unfamiliar fruits. A row of stalls sells medicines and charms — herbal oils, lizard skulls, alligators' teeth, dolphins' penises, turtle shells, and blue-and-white banded snakes, with milky, opal eyes, suspended in jars of fluid. Close by, a little shop sells the curious paraphernelia of the African-derived Umbanda religion, which includes among its pantheon St George (complete in shield and armour, slaying the dragon), Iemanjá, white-robed African goddess of the sea, and Pai Joaô, a cigar-smoking black slave.

Even the ice creams are Amazonian; alongside the familiar tropical tastes of pineapple, guava and mango are açai, bacuri, caju, cupuaçu, muruci, graviola and genipapo*. Several of these fruits are collected from the forest and not yet grown in cultivation. Even so, it is just a matter of time before they reach New York: the agricultural research station in Belém has worked out how to spin-dry and powder some of the fruits for long-term storage.

Belém is just south of the Equator, 2,000 miles north of Rio de Janeiro and 80 miles from the open sea. It is the capital of Pará, a state almost six times the size of Great Britain. Although an exotic place for the visitor, Belém is also the home of three of the most important scientific institutions within Amazônia: the Emilio Goeldi Museum, which is the region's oldest scientific institution, the Federal University of Pará, and the Centre for Agricultural Research in the Humid Tropics (Centro de Pesquisa Agropecuária do Tropico Úmido, CPATU). The heads of all three institutions have much on their minds — the parlous state of their finances, politics, their research plans and, not surprisingly, the emergence of the Amazon as a world issue. On this latter issue they all agree that rational, sustainable development of the Amazon is the only way forward (not the irrational development of some of Brazil's giant projects, nor the rational non-development of diehard conservationists), but they disagree on other points, particularly on how well Brazil has done so far and on the role foreigners might play. □

*These fruits are *Euterpe oleracea, Platonia insignis, Anacardium occidentale, Theobroma grandiflorum, Byrsonima crassifolia, Annona muricata* and *Genipa americana*. Some are found only in the Amazon region, others are cultivated throughout the tropics.

An Amazon University for Amazônia

NILSON Pinto Oliveira is rector of the Federal University of Pará, which occupies a campus down by the river on the outskirts of Belém. The university would not come on anyone's list of the best universities in Brazil, but it is the best in the Amazon — and not just the Brazilian Amazon, but the whole region.

Oliveira knows the potential of the region and the reality of his resources. The university has 1,500 faculty but just 135 with doctoral degrees — one trained researcher for every 36,000 square kilometres of the Amazon. But as Oliveira says, there is no time to stop, "the Amazon is not simply a place to admire, its a country where people live and will continue to live. The people are poor, they need money for the next day."

Oliveira has been rector for three months and clearly chafes at the many restrictions under which he has to operate. His first priority is to raise the level of training of the university staff and help improve Amazon educational standards. "We cannot talk about the protection of the Amazon region if we do not give to the Amazon people the possibility of learning and understanding their problems", he says. The university, along with other institutions in the region, is running a scheme to retrain school teachers. At sites throughout the Amazon, some 2,000 schoolteachers come to twice-yearly three-month courses. Raising university standards is exceedingly difficult. Most of the faculty, perhaps 90 per cent, do no research but simply attend the university to teach. A considerable number bend the rules and hold other jobs elsewhere. The four-year-old freeze on appointments prevents new staff being brought into the university. And central control by the Ministry of Education prevents change.

If Oliveira had his way, he would prefer 500 well-trained researchers than the 1,500 staff he has now. He sees lack of flexibility as an even bigger problem than inflation or shortage of money. "All universities are considered to be the same by the ministry", he says, "But the priorities here are completely different from the University of Rio de Janeiro. I cannot create posts in the area they are needed."

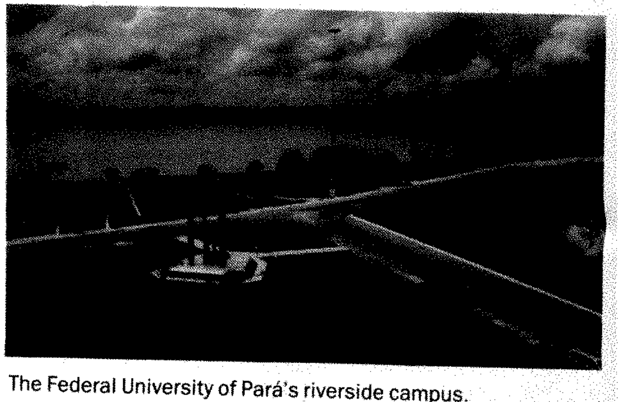
The university hopes to consolidate its resources in environmental research in a Centre for Ecological Science. Foreign help is needed to train people. But Oliveira, like others in Brazil, is convinced that foreign help must be true cooperation which will build up expertise i the Amazon.

Perhaps 70 to 80 people at the university are involved in research. Geoscience is one active area and is involved in a collobortive project funded by the US NSF to study the Amazon shelf off Marajó Island. Another active basic research group, in primate genetics and evolution, is run by the husband-and-wife team of Horacio and Maria Cruz Schneider.

Oliveira: making the best of things.

Their research has received a bonus from the construction of some of the Amazon's controversial hydroelectric dams. When the reservoirs are flooded, power companies are under obligation t catch large animals trapped by the risin waters and release them elsewhere. The Schneiders collect blood samples from captured monkeys. Study of blood groups, serum proteins and chromosome types enables questions of New World primate speciation and evolution to be tackled.

The Schneiders' efforts are quite heroic. The laboratory roof sometimes leaks, the water and electricity supply are unreliable, and 90 per cent of their electrophoretic reagents have to be imported with all the delay and bureaucratic uncertainities that involves. The university is too poor to afford journal subscriptions. Instead, the primate group has banded together to pay for airmail delivery of *Current Contents*. They then write for reprints to the authors of relevant work. The team has great spirit; all 15 primate researchers are young and are committed to refusing any side jobs. They are also building their international links; members of the group are slowly being 'strategically placed' on fellowships at universities in the United States, Japan and Europe. □


The Federal University of Pará's riverside campus.

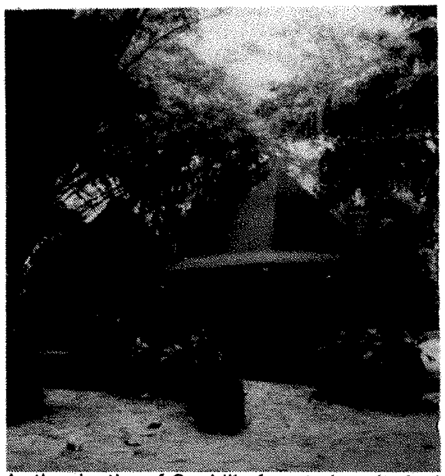
Museum ups and down

GUILHERME de La Penha is director of the Museu Paraense Emilio Goeldi, to give it its full title. The museum is old, established in 1866, and looks as though it has emerged from the pages of a Persian miniature painting. In a densely wooded, walled botanical garden are set pools and aviaries full of the birds and beasts of the Amazon: manatees, jaguars, giant river otters, cranes and toucans — 600 species in all. Almost half a million visitors come here each year. Its scientific heyday was early in the century when the Swiss zoologist Emil August Goeldi was director and began its collections of biological, ethnographic, archaeological and geological specimens.

The past few years have seen a revival at the museum, for which de La Penha can claim some credit. The museum is quite small, with around 100 scientists, and has been able to adapt to changing cirumstances more quickly than INPA, the much larger Amazon research institute in Manaus.

De La Penha says some 30 or so of the staff are now producing "good science", and have won back an international reputation for the museum. Both the Ford Foundation and the MacArthur Foundation are supporting research there and more than 40 people have been sent away for postdoctoral training. The hope is that their gradual return will take the museum to a much higher level. The museum's rising fortunes were celebrated in October with the grant of 30,000 hectares of natural forest at the junctions of the Xingu and Amazon rivers. A new field research station is to be equipped, most probably with help stemming from an environmental memorandum of understanding signed between Great Britain and Brazil earlier this year. The agreement was a nice coup for the British Embassy's diplomats, having been signed at the height of Brazil's annoyance with foreign 'interference' in its Amazonian policy.

But that is the good news. The bad news is that the museum is very hard hit by this year's cuts in funding for science. De La Penha appears under siege. "We cannot buy equipment, we cannot buy books. Everything that is important and imported has become impossible for us", he says. Inflation has also taken its toll." The value of salaries has fallen so much that most scientists are living on their savings", he says. "Now we're always worried about how we will reach the end of the month. You want some peace to publish manuscripts, but you go to bed wondering how can you survive."



In the depths of Goeldi's forested zoological garden.

Even in Belém, Brazil's political turmoil is felt. The museum is one of CNPq's institutes and CNPq has come out against the idea that all members of an institute — including the large numbers of support staff — should have an equal vote in deciding how it is run and who runs it. But depending on the presidential election, 'democracy' may triumph. De La Penha is disgusted with the intrusion of politics into science. "Incompetents are reaching high positions in the universities and institutes", he says, "although there is meant to be evaluation by peers, peers are afraid the person they evaluate may prove to belong to the right political party. If you're popular you get elected, it doesn't matter if you're competent, you must be popular with students, clerks. . .".

De La Penha is not a stranger to political fights. He left the country in 1983 after quarrels over planning for research in Amazônia. He had been vice-president of CNPq and organized a meeting in Santarem to discuss a plan for development of the Amazon. "Even today it is the only written plan for research in the Amazon", he says, "then we tried to put on pressure to create a tropical research programme, we also criticized the Interior Ministry, showing that many of its project funds just supported the infrastructure of institutes and were not used for research." These and other activities got de La Penha into hot water, and he accepted 'exile' to a job at the Organization of American States in Washington, DC. But two years ago he returned to Belém. His aim now is to establish a small, very strong scientific institute and not to interfere with the Amazon development agencies. "If it is strong it will be heard", he says,"and will provide hints, not solutions, of how to develop the Amazon on a rational basis. The Amazon is a complicated set of limited regions that need to be looked after differently, you cannot have a theory of the whole Amazon."

Foreign collaboration is bound to play a big part in the study of the Amazon, says de La Penha. He laughs at politicians who "every time you talk about foreign cooperation in the Amazon, say its a foreign trick". "We have to have added cooperation. Most of the research on the Amazon is still published by US and UK scientists. They have better continuity and can carry on a project for years. There are segments of Brazilian science who say, leave the Amazon to Brazilian scientists. But that would be one scientist for how many thousand square kilometres? Brazilian scientists must enter the international community. You cannot learn if you attend only regional conferences."

But foreigners who have jumped on the rain-forest bandwaggon are another matter. He wishes that some of the 'Save the Rain Forest' groups in the United States would spend their money in Brazil where it is urgently needed, instead of on "parties and lobbying" in Washington.

The museum is desperate for the most basic equipment. De La Penha managed to buy up one truck load of glass containers that were not good enough for the Smithsonian Institution's needs. He hopes to get another, and dreams of good microscopes and, even more, of personal computers. ☐

Short shrift for foreign do-gooders

ASKING CPATU chief Emeleocípio Botelho de Andrade about his views on foreign interest in conserving the Amazon provokes an explosion. There is "nothing but emotion, disinformation and economic interest" behind international criticism of Brazil's conduct, he says, "foreign powers are afraid that Brazil will develop as quickly as Japan or West Germany".

CPATU's job is to help speed the agricultural development of Brazil's humid tropical regions, so Botelho de Andrade's annoyance over foreign criticism is understandable. The institute is one of the 27 research stations — each responsible for a particular product or agricultural zone — belonging to EMBRAPA, the agriculture ministry's research organization.

Energy policy is a prime example of foreign perfidy, in Botelho de Andrade's view. West Germany and the United States have taken $8,000 million from Brazil for the development of nuclear power plants with almost no return on the investment, he says. But when Brazil wants to develop hydroelectric power, the cheap and logical energy source for Brazil, the same nations stir up problems over the Indian peoples who live at dam sites and try to block Brazil's development.

He claims the foreign view of the agricultural capabilities of the Amazon is too pessimistic. The Amazon's soil and vegetation types were mapped in a huge radar project (RADAM) a decade ago. Much of Rondônia, where forest burning has provoked greatest outrage, has fertile soil capable of sustaining agriculture, he says. Overall, he estimates that 8 per cent of the Amazon will prove to be highly fertile, an papers. This year, for the first time, the group will pick up substantial funding inside the country, from the Bank of Brazil. The funds will provide for a project on mercury pollution of rivers from gold-mining operations by unlicensed prospectors. A preliminary study on the River Madeira in Rondônia has already found dangerous levels of mercury in three locally eaten kinds of fish.

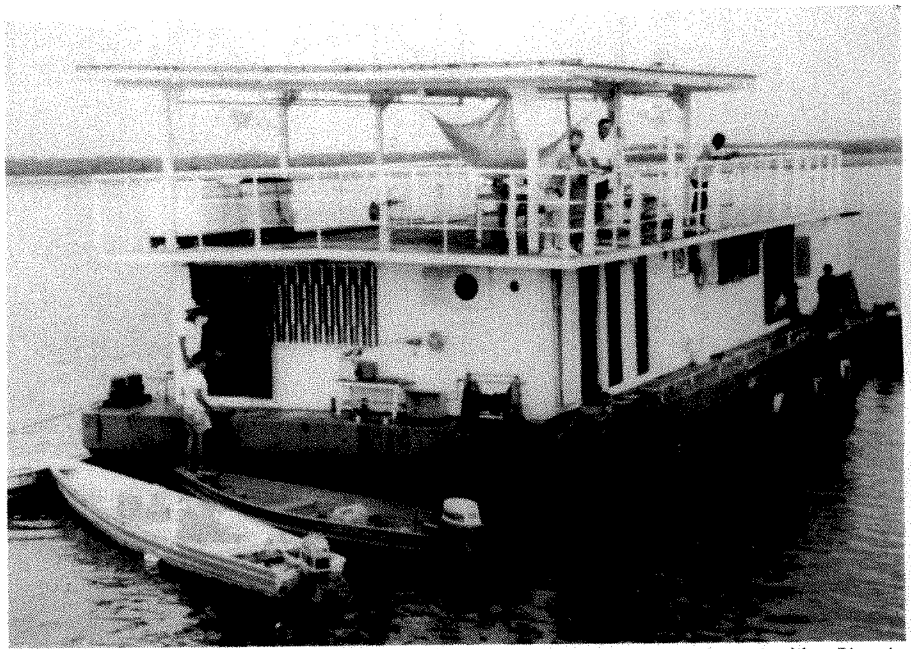
Like several other foreigners at INPA, Forsberg has been able to become an official employee. He has a tiny office, about the size of an American cupboard, in the centre of which he perches on a curious home-made stool, apparently of his own invention. Phillip Fearnside is another foreign staff member and the biggest star internationally. His work on human settlement in the Amazon and his critiques of the giant development projects — the TransAmazon highway, the Balbina dam, Ludwig's Jari colony and the Carajás smelting project — have turned him from lone academic to international spokesman. To his credit, Fearnside's studies have won tremendous respect inside Brazil; despite his criticism of many government projects, he is not regarded as a mere interfering foreigner. Fearnside, a very tall and very thin American, with a moustache that would be the envy of a walrus, is still a quiet academic. But he seems to enjoy the fact that complete strangers sometimes recognize him from his televison appearances, even in the wilds of Rondônia.

That foreigners can become employees, and that they can apply for Brazilian research grants, is evidence of open-mindedness on the part of the Brazilian scientific community. But that foreign researchers sometimes obtain both Brazilian and foreign funds can also provoke envy. And regrettably, some insensitive foreigners have come here like colonists of old, completing their projects and departing, leaving nothing behind for their hosts. INPA is considering whether it should begin charging overheads to visitors.

Brasilía too is sometimes heavy-handed. Foreign collaborations do require a complete, matching set of Brazilian and foreign partners, but application of the rules is often cumbersome and has slowed good projects.

Foreigners are luckily immune from the institute's politics, of which there is far too much. Among its most senior people are some with poor scientific credentials whose ascendancy owes much to good political connections. But that perhaps just adds another dimension to an institute whose main quality is enormous diversity in approach, quality and style. There are researchers — both Brazilian and foreign — with an international reputation and others who rarely publish, even in an obscure local journal. There are researchers such as Ilse Walker, who is as happy arguing about population biology or Eigen's hypercycle theory (or anything else) as she is on the river collecting zooplankton, her speciality. Others appear not to have read an international journal in a decade. And there is the mix of people pursuing quite practical problems, such as the suitability of Amazon timber for building, and those whose interests are in basic research, modelling energy flows in ecosystems, for example.

Such heterogeneity may be both stimulating and reveal a weakness: in a visit of only a couple of days, it is possible to uncover researchers who have never heard of one another, even though their work has considerable common ground. That surely suggests a loss of common purpose. Perhaps it is time someone began to organize some institutional seminars. □



Boat people. The well equipped floating laboratory, crewed by scientists from the Max-Planck-Institute (see below).

Almost too many species to count

Close to Manaus, the Rio Negro is several miles wide. Out in a boat the eye is drawn not to the river, nor to the green line of the forest, but to the sky, a vast sky like that over the ocean or the prairies. Over the day, a series of giant clouds form, build into fantastic storm shapes and then resolve themselves in short, torrential rain showers (see following page). Here the Amazon is flat and the views distant; the river is descending towards the sea at a mere metre every 100 km.

Upstream is the Anavilhanas, the world's largest freshwater archipelago, where the Rio Negro divides into a myriad channels. Downstream a few miles the black waters of the Rio Negro join the pale brown waters of the Rio Solimões. The two flow together for miles, mixing only slowly to form the Amazon. Freshwater dolphin live here and follow passing boats; one species is a curious shade of shocking pink.

A little distance up the Solimões, anchored in a channel away from the main river, is a floating laboratory run by an INPA-based group from the Max-Planck-Institute for Limnology at Plön. It is a meditative spot, the silence broken only by the cry of birds (some of which have a startling resemblance to the cormorants and kingfishers of Europe). Small boats pass by now and again in a larger channel, destination unknown. One researcher speculates out loud if it might be nice just to buy a boat and set off wandering up the Amazon.

There is a host of interesting problems that can be studied from here, including the hydrology and chemistry of the river itself. But many researchers have turned to the interaction between the river and the land, which is both more visible and more complex than in temperate zones.

The banks are muddy, not because there are tides, but because the river may fall as much as 15 metres between the high season in June and the low in November. Each year, a cycle is repeated in which shore-dwelling plants and animals are first inundated and then left high and dry, and river fish move in beneath the flooded forest and then out again with the retreating flood waters. The result is a unique environment in which plants and animals must adapt to both land and water phases. There are fish that live exclusively on the fruit falling from the forests, others that breathe air to get them through the flood phase when organic matter is decomposgradually run down? The researchers do not know yet, but the project is intended to continue for another 20 years. Along the way, the project should provide many data to test theories of island biogeography and come up with some practical idea on how groups of Amazon species can be maintained. □

INPE-SPACE INSTITUTE

At the centre of a storm

São Jose dos Campos

It is a curious circumstance that if it were not for a quarrel over deforestation of the Amazon, the National Institute of Space Research (INPE) would be little known abroad. International recognition, or at least the attention of the international media, came to the institute ealier this year when President José Sarney claimed that satellite data analysed at the institute showed that a mere 5.12 per cent of the Amazon had been cut down since 1500. In the quarrel over the validity of the figures that followed (see *Nature* **339**, 86; 1989), the world press descended on the institute.

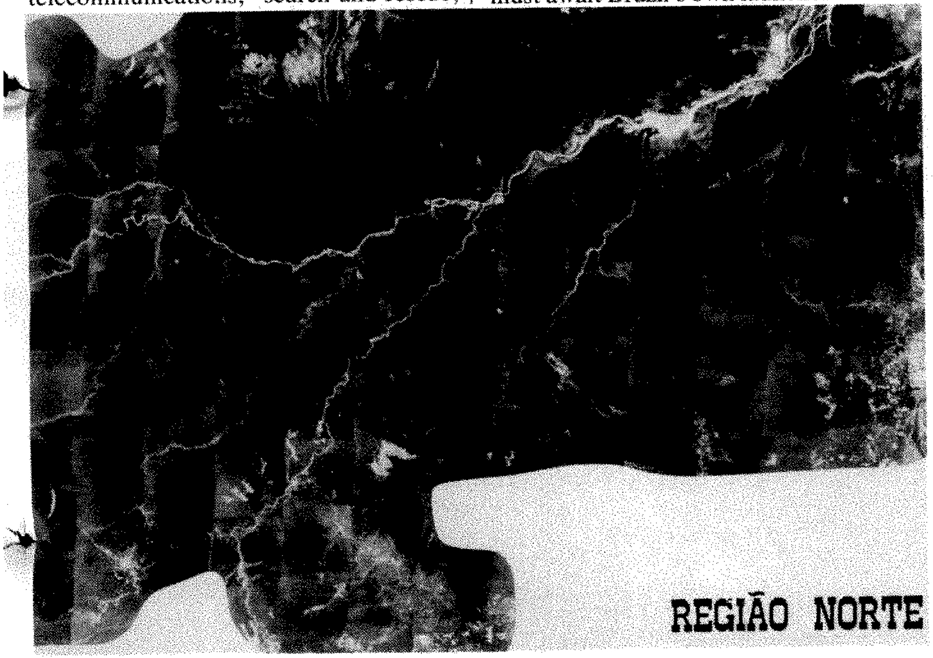
Remote sensing is in fact only one part of what INPE does. It is Brazil's biggest single research institute, employing more than 1,700 researchers in the fields of atmospheric science, astrophysics, space science and meteorology. The institute is responsible for Brazil's satellite programme and will soon see the completion of the nation's first indigenous satellite, SCD-1, being fabricated at the aeronautics company Embraer to INPE's specification.

The 115-kg SCD-1 is a relatively unsophisticated satellite, designed to collect meteorological data from 10 land stations at remote sites across the nation and retransmit them to a receiving station in Mato Grosso. But the satellite is also a key engineering test bed. By 1995, Brazil plans to launch seven satellites for use in telecommunications, search-and-rescue, meteorology and exploration for mineral resources. That, at least, is the plan.

A major difficulty is that a separate programme to develop rocket launchers being carried out by the Space Activities Institute of the Aeronautics Ministry is way behind schedule. A one-third size scaled-down version of the rocket was launched successfully last May but there is little chance that the full-scale rocket can be ready before mid-1992, more than two years after INPE could have its satellite complete.

The rocket programme has been held up partly because of the unwillingness of the industrialized powers to transfer technology to a programme that is controlled by the military and could easily be diverted for use in long-range ballistic missiles. US refusal to sell inertial guidance systems to Brazil triggered a minor diplomatic row in April. But things are unlikely to change, given that Brazil is an important arms supplier to developing nations and provided rockets, tanks and armoured cars in the recent Middle East conflict.

Many within INPE would like to see a foreign launcher used to put the first satellites into orbit and so keep their part of the programme on schedule. A separate agreement has already been reached to build a remote-sensing satellite for China and launch it on a Chinese rocket. But the military insist that Brazil's programme must await Brazil's own launcher.



The Amazon basin in mosaic form from INPE. The fine grid of lines at the bottom is the road system in Rondônia. Around it much of the forest has been cut down.

Bitter about media treatment, remote sensing director da Cunha.

Meanwhile, Roberto Pereira da Cunha, director of remote sensing at INPE, still feels bitter about press handling of the dispute that thrust prominence on INPE last April. The project was important because it was the first complete satellite image study of deforestation in a decade. But the message was lost amid controversy over the figures.

"The problem is that everyone likes to do sophisticated things", says da Cunha, "everyone likes to talk about the influence of deforestation on climate and global warming but no one wants to do the dirty work of gathering the data. It is a very trivial job for scientists." Deforestation estimates varied from 44 per cent to the World Bank's 12 per cent, but all were projections from old figures. INPE scientists calculated from a mosaic of 101 Landsat images that in fact 5.12 per cent of the government-defined 'Legal Amazon' had been deforested in recent times (not since 1500 as Sarney claimed — there are large areas of very old deforestation).

Arguments continue over whether deforestation should be related to the Legal Amazon, or to the total area of rainforest or some other number but da Cunha says these are not really important.

Absolute values matter little compared with continuing rates of deforestation. He is now trying to obtain funds to study changes through to 1992 as part of the International Space Year, in collaboration with the US National Aeronautics and Space Administration and the European Space Agency.

Da Cunha stresses that remote sensing has many uses and his group, almost 200 strong, puts much of its effort into research in image-processing and generation as well as applications research and technology transfer. Satellite data have been used to settle legal disputes over whether land is in use or not (a key factor in maintaining ownership), to estimate the areas of flooding of the giant reservoirs and to monitor development projects. And in a more esoteric project, some of da Cunha's group are using satellite data to study the gigantic and enigmatic patterns of lines in the desert at Nacza in Peru, to see if they were drawn according to astronomical principles. □

UNIVERSITY OF SÃO PAULO

The biggest and the best

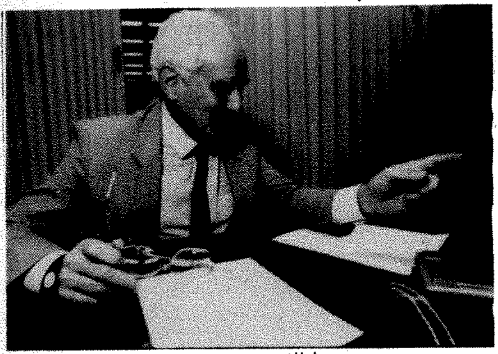
São Paulo

IT is a cruel shock to the senses to arrive in São Paulo on a wet, dark night. All that can be seen is a spinning confusion of freeways, underpasses, missed turnoffs and high-speed drivers who overtake on the inside and cut in front of one with a little flourish. São Paulo is huge; ringed by factories and slums and dissected by an irrevocably polluted pair of rivers. With more than nine million inhabitants, it is the largest city in Latin America and the third largest in the world.

Surprisingly, dawn reveals that São Paulo is also beautiful. It is a hilly city with plenty of green spaces and views and the broken skyline of tower blocks is never monotonous. Out of town — at around the 20-kilometre mark — the city degenerates sharply into shacks and shabby shanty towns, home to five million of the poorest.

São Paulo is the capital of the richest state in Brazil. Its inhabitants add up to less than a quarter of the total population but they generate half the nation's GNP. This one state contains half of Brazil's top eight universities, and best of them all is the University of São Paulo (USP).

The university is a little out of town, scattered over a slope from the top of which São Paulo is seen spreading across the horizon. At the bottom of the hill is a clock tower and the other usual symbols of a university campus and, quite out of place, a two-km-long rowing tank. This splendid facility was apparently built for a Pan-American games that was never held.



Folha de São Paulo

Goldemberg: centre-stage until January.

Ruler of this university for the past four years has been physicist José Goldemberg, a larger than life figure well known both as rector and for his criticism of the government's nuclear programme (see page 374). His term of office ends in January.

The university is big: 35,000 undergraduate students, 15,000 graduate students (half the doctoral degrees in Brazil are taken here), 5,000 faculty and 15,000 support staff. Eighty per cent of students study science, engineering or medicine. Seventy per cent of the faculty hold doctorates, the highest percentage in Brazil.

Goldemberg has been a controversial rector who shook up the university through insisting on increased emphasis on research.

When he took office, the university had few funds of its own and almost all the money for research, even down to journal subscriptions, came from grants. Now 20 per cent of the university's $250 million-a-year income from São Paulo state goes on non-personnel costs — laboratories, computers, libraries and the like.

Goldemberg says that when he became rector he decided to "make great efforts to be in the vanguard. Since Brazil needs modern technology we have to put a lot of emphasis on research and high quality." He says he met resistance.

"How do you put pressure on people who all have tenure?" he asks rhetorically. "You use a complex sytem of incentives — access to money, and better libraries, better communications, computers, sending people abroad . . .". Goldemberg recounts his various temptations and sighs over those who were not ready for them.

"Some areas were not in a position to absorb money. They had no ideas, no contacts. I would tell people to make a list of everything they wanted, then they found they could not make a proper list, they were used to underdevelopment." "People thought I was a bit harsh", he says, "but after the event they thought it was not so bad and even approved of what I had done."

Goldemberg's successor, Roberto Leal Lobo, was chosen by election two weeks ago. As elsewhere, there was heated debate over how much say students and support staff should have in the new 'democratic' election. USP decided on a safe multi-stage election that left the final choice to the professors and, ultimately, the governor.

Lobo is a physicist and was previously vice-rector, but his election was not a foregone conclusion. Running against him were the Secretary of State for Science and Technology (page 371), and half a dozen other university professors. A key issue during the campaign was the university's links with industry. Some candidates were against expansion of industrial involvement, others wanted it to be given the highest priority. Lobo backed a middle way with, he says, "human resources as the first priority, with an overflow to industry. Otherwise we'll just become an industrial research institute."

'Evaluation' was another controversial issue in the election. Many faculty wanted the cosy option of evaluation being left within departments, but Lobo backs the move to more independent assessment. A doctorate is essential now for a staff position and Lobo claims that contracts of non-tenured staff are no longer automatically renewed. Although there is no comparison with the driving pressure of the United States, USP is moving towards a more competitive environment.

Facilities at USP are the best in Brazil and will soon be even better thanks to a $150 million loan from the InterAmerican Development Bank (IDB). The sophistication of the university allows researchers to keep up with international trends to an extent not visible at other Brazilian universities.

Researchers at the Institute of Physics, for example, were quick to replicate experiments demonstrating high-temperature superconductivity. They also jumped in immediately when they heard about cold fusion, but director Ivan Cunha Nascimento says that "after three months we decided to stick with high-temperature fusion!" The institute has a plasma-physics group and a small tokamak, the only one in Latin America. IDB funds have provided for a new molecular beam epitaxy laboratory and two new electron microscopes.

Elsewhere, the Institute of Oceanography is able to run yearly expeditions to the Antarctic where Brazil maintains an all-year-round base. That gives the geologists from the Institute of Geosciences a

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UNIVERSITY OF CAMPINAS

Technology the driving force

Campinas

THE State University of Campinas, located in São Paulo state about 100 km from the capital, is often described as "second only to USP". But it is not an imitator of USP. From the time it began life in 1966, Campinas chose to give priority to science, technological research and, uniquely, links to the production sector. The first courses it offered were in physics, chemistry and mathematics, set alongside technological projects in optical fibres. lasers and computers. Next came engineering and last of all, in the 1980s, the arts.

Campinas now offers a full range of disciplines. It is very popular and has around 23 applicants for every place, making it more competitive even than USP, although it is only half the size. The campus has a quiet feel, set amid sugar cane fields in the rolling countryside a few miles outside the city of Campinas, the second largest in São Paulo state with a population of three-quarters of a million. Although the university was created at the height of the university expansion boom, it managed to keep to a policy of recruiting only the best staff. Sixty per cent of faculty hold doctorates, which is a very high proportion in Brazil, and around ten per cent are from foreign universities.

Campinas faculty like to list their university's unique features. First, almost half of its

,000 students are pursuing graduate courses. any are themselves teachers on release om other universities all over Brazil, giving pinas the reputation of a 'school for ools'. Second, researchers are encouraged build their own links with industry and en to profit from them. Campinas was the st university to set up a patent office to otect its staff's discoveries (a move which s still found few imitators). Several searchers have set up their own small mpanies. Third, the university has been gressive in buying good facilities.

At the the end of last year, it bought an M 3090, giving it the most powerful supermputer in Latin America. Next, as soon as deral funds are available, will come the ational Synchrotron Light Facility. From e beginning it has had the National Center r Research in Telecommunications.

In 1986 it created a large biotechnology stitute at the stroke of a pen by buying arby Monsanto Corporation's Agricultural esearch Center. Later, it complemented e centre with a Genetic and Molecular ngineering Center to train people for the pected expansion in biotechnology. Now ere is an multidisciplinary programme tred around biotechnology, alongside nilar integrated schemes in computers, fine emicals, energy and new materials. The ctor, Paulo Renato Costa Souza, says that s univerity's philosophy of trying to build tegrated programmes, instead of letting eryone submit individual grants, is "cometely new in the university system". ☐

BUTANTAN

Biomedicine with a bite

São Paulo

Snakes, scorpions and spiders make the Institute Butantan famous. Its serpentarium is São Paulo's most popular tourist attraction and every year thousands of people from all over Brazil send to the institute snakes and other exotic creatures they have found in field or garden.

The snakes are essential for Butantan's function as the main supplier of antivenom in Brazil. Venom is milked from the snakes and used to hyperimmunize horses (the institute keeps 700 at stables outside São Paulo); a specific gamma globulin fraction containing antibodies against the venom is later extracted from the horses' blood. The institute maintains a small hospital, manned 24 hours a day, with a helicopter pad in front of it.

People bitten in the São Paulo region are rushed here — treatment within the first three hours is most effective. Brazil has an impressive array of poisonous snakes and more than 30,000 people are bitten each year.

Snakes are not the institute's only concern. Butantan is essentially a large biomedical research institute joined with a production facility. As well as snake antivenoms, it produces 16 kinds of vaccine. Its 1,300 staff are divided among 20 different departments of basic research and a new Centre of Biotechnology.

Naturally enough, some of its key projects involve improving vaccines. One involves cloning polio virus in vaccinia with the eventual aim of constructing a single-shot vaccine cocktail for polio, measles, hepatitis B, diphtheria, tetanus and whooping cough. Another is to develop a genetically engineered hepatitis-B vaccine, although the institute may decide to buy the technology from abroad. Projects to produce albumin and factor VIII by new techniques are also under way.

Other groups do basic research on venoms (the important vasodilator drug bradykinin was first isolated here), viruses (it is still relatively easy to discover a new virus a day in the rain forest), the genetics of amphibians and reptiles, and the isolation of natural substances from plants and marine animals.

Techniques developed at the institute are transferred free "to anyone seriously interested in production", says the director, Willy Beçak. "We're not interested in profit but to be in the at the frontier", he says; "the idea is for the institute to be an open place for people from universities; often universities are not the right place for transfer to the production sector."

Folha de São Paulo

Willy Beçak and one of his Butantan production workers.

The institute's popularity rose after it opened a 'reagent supermarket' in an effort to counter the effect of import restrictions.

Special permission to import reagents tax-free in bulk, together with a $1 million grant from CNPq, enables Butantan to offer immediate delivery of a range of hard-to-import items. But it is not permitted to duplicate reagents manufactured in Brazil. ☐

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chance to examine structures in Antarctica to which Brazil was once joined. Overall, the impression at USP is of a university that is only occasionally at the international frontiers but is frequently not far behind them.

But like researchers elsewhere, USP staff are held back by petty import restrictions. Some laboratories — the Institute of Chemistry is one — employ several people simply to do the paperwork. But the delay remains. Ana Clara Schenberg, a yeast geneticist at the Institute of Biomedical Sciences, has been waiting for one piece of equipment for four years. It is already obsolete.

Life is particularly difficult for molecular biologists. Even things such as dry ice are in short supply and items that need to be used quickly — short-lived isotopes, for example — are almost unobtainable. And, worst of all, delay destroys flexibility. "If you need to change your research programme, just forget it", says molecular biologist Bianca Zingales. Everything has to be planned ahead around the import of reagents.

Many highly trained young researchers are now returning from abroad, thanks to earlier government decisions to invest in foreign fellowships, offering the chance to push the university ahead. Unfortunately, the returning researchers often seem quite lost — particularly those who have been at top US laboratories.

Around the campus, young people in various stages of 'returnee's syndrome' can be met. Salete Newton, who returned from a postdoctoral fellowship at Stanford University three months ago, is in the fairly early stages. "You don't know what to do", she says, "you know everything will take ten times longer than in America. There the limit is your imagination, here you are trapped by the limitations of the facilities."

A little later, the returnee struggles to see advantages. "At last I have time to read all those papers", says one. "Maybe it's a good thing that I'm forced to think instead of just rushing onto the obvious experiment", says another. Along comes the thought that one is free.

"I realized I could try some stuff that would be thought too risky over there, the sort of thing you would only do if you are already a full professor", says molecular biologist Fernando Reinach, who has no symptoms of the syndrome after three years back home. Returnees also come to realize that at USP one can attract the very best students of one's own.

The fully recovered researcher avoids the most internationally competitive areas but is busy inviting foreign colleagues for short stays (provided they agree to smuggle restriction enzymes, short-lived isotopes and the like through the Brazilian customs), using BITNET and facsimile machines to keep up, building a group in an area of his or her own, and sending off students for doctoral training abroad. Of course, there are always a few who never recover and just settle back. . . . ☐

OSWALDO CRUZ

Institute earns healthy respect

Rio de Janeiro

THE Oswaldo Cruz Foundation's grounds are ringed with fences, walls and armed guards who harbour deep suspicions about strangers. Presentation of a copy of *Nature* will in no way convince them of one's good intentions. That and the baroque architecture of the 'Moorish Pavilion', set on a hill amid dark trees above, suggests one may have accidentally stumbled on a secret military organization rather than a biomedical foundation.

Later, an explanation emerges. The foundation, on the northern outskirts of Rio de Janeiro, is ringed with *favelas*. A few weeks earlier a couple of men dressed as policemen had come along and persuaded a naive academic to loan them his car for an 'exercise'. They then robbed the bank on the foundation's grounds and vanished (or so the story goes).

J. Pinto

Imposing prospect: the Oswaldo Cruz Institute is important politically as well as medically.

The normally peaceful institute was set up by Oswaldo Cruz in 1900 to produce vaccines to combat local epidemics of plague and yellow fever. It still produces vaccines (like its sister Institute Butantan, page 369) and over the years has gained a reputation as the most distinguished biomedical research institute in Brazil, with a considerable voice in public affairs.

In its grounds is the National Public Health School which trains most of the nation's health administrators. Akira Homma, the institute's director, is clearly proud that the institute helped to write Brazil's new constitution.

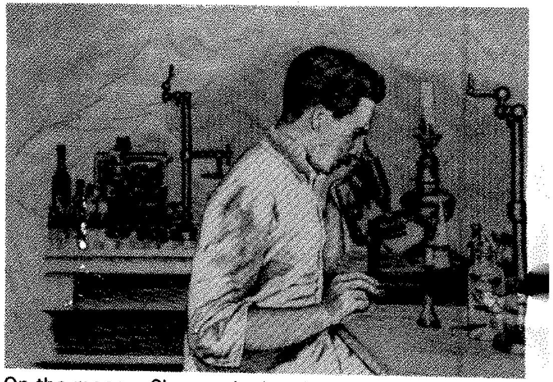
Half the institute's budget comes from the government and a third from the sale of vaccines. Vaccine sales are important, says Homma, because that means "we have our own budget, produced by ourselves which we can invest by ourselves". Production is being built up further with national self-sufficiency the goal. Polio vaccine is still all imported but the institute supplies much of the world's yellow-fever vaccine.

The production facilities aside, the institute feels little different from a university. But the focus is on "endemic diseases and their vectors", research director Carlos Médicis Morel explains, with the link between research and production clearly in mind. The institute's 200 researchers are expected to publish in international journals and to be capable of true international collaboration — "Not the old kind where we have the diseases and they have the technology", says Morel.

Grants from the World Health Organization provide foreign currency and allow some items to be obtained on a 'diplomatic' basis through the Pan American Health Foundation. The result is a miracle in Brazil — Morel is able to order short-lived isotopes by telex from abroad and see them delivered in 24 hours. "There is a fight for every microlitre", he says.

A new biotechnology department is to combine research and pilot plant production and will concentrate on diseases transferred by the blood — including AIDS, malaria, syphilis and, of course, Chagas' disease.

Chagas' disease, named after the former director, is still both an important research topic at the institute and a major health problem in Latin America. As many as 10–12 million people are affected. Modern medicine has not cured the illness but has brought new modes of transmission. In Brazil, 20,000 people a year are infected through contaminated blood transfusions, perhaps more than the number infected by insect bites. Despite the scale of the problem little was done until AIDS arrived. Previously, poor people were commonly selling blood to private transfusion banks. That is now illegal (thanks in part to the institute's lobbying). But the change has come too late for haemophiliacs. Sadly, 70 per cent of them carry the AIDS virus.



On the money: Chagas depicted on a 10,000 cruzados banknote.

Morel, when not wearing his research director's hat, runs a group developing DNA probes for diagnosis of Chagas' disease. Samuel Goldenberg, a young molecular biologist returned from Paris, is trying a different approach, using recombinant antigens.

Diagnostic kits in use now cross-react with antigens of other diseases. His group has cloned several *Trypanosoma cruzi* surface antigen genes, and one has good potential for use in a test kit.

Goldenberg has a fine sense of the differences between laboratories here and in Paris and the pains and joys of working in Brazil. His field is the molecular biology of development.

"Elsewhere", he says, "I would probably work with *Dictostylium*. Here I work with trypanosomes. My main interest was in basic biology. but now we may have an application for our work. When you feel your science is being used for something useful its very very good."

His group has found factors that trigger the switch from the nonreplicative stage of *T. cruzi* to the replicative stage and is looking for enzymes important in the differentiation process. If enzymes unique to the trypanosome can be found, therapy with specific inhibitors may be possible.

Goldenberg's laboratory is overcrowded to bursting point. The corridors are so full of equipment that if two people meet they have to work out a way of backing into the remaining spaces in order to get past one another. If three or more people meet, the moves become just too complex — it is better just to return to one's room.

Goldenberg says that he sometimes "gets really fed up with the difficulties", that "he cannot hire new staff but is simply sending people abroad", that the strict importation rules mean that he is "treated like a smuggler" and that foreign journals come by sea, "three or four months late". But, he says, "the ambition here is not to publish, publish, publish as in the United States — we do want to publish but the main goal is to train more people".

In mid-October, Goldenberg was busy trying to organize the 16th Annual Meeting on Chagas' disease and leishmaniasis, due to begin a few weeks later at a resort hotel. Six hundred participants were expected. The organizers pay for research students' accommodation, "otherwise the students won't be able to go", says Goldenberg, "and its a good opportunity for them to meet people, find a place to do a postdoc. But it is a hard task because we cannot find any funds!" When last seen, Goldenberg was listening to the manager of the hotel shouting, "When will you send the money. When will you send the money?" over the telephone. In Brazil there usually seems to be some way to fix the impossible — no doubt the conference went well. □

SCIENCE MINISTER

Informatics law and disorder

São Paulo

DÉCIO Leal Zagottis is Secretary of State of the Special Secretariat for Science and Technology of the President of the Republic of Brazil. Had he taken office last year rather than this, he could have done the same job and been simply Minister of Science and Technology; the cumbersome title is the result of a political battle for control of the ministry during the first few months of this year.

The opening broadside came in January with the sudden announcement that the Ministry of Science and Technology was to be abolished and its functions shared out among other ministries.

At the same time, the president agreed to wrenching cutbacks that reduced science and technology budgets by half and would have led to the dismissal of 40 per cent of research staff at CNPq institutes. But the surprise attack was motivated not just by the desire to save money; in the vanguard of the charge were those who opposed the ministry's controversial informatics law.

As it turned out, the abolitionists did not prevail. Congress rallied to the ministry's cause and three months later it was back, albeit under a new title which left it with a little less weight than a full ministry. And Zagottis, whose support for the informatics law is well known, was appointed to head the new agency.

The controversial law was passed in 1984 to foster the local production of computers, software and peripherals by reserving the Brazilian market for 'national' companies, that is, those that are Brazilian-managed and at least 70 per cent Brazilian-owned. The law has been bitterly opposed, both by the United States which sees it as restricting access by its giant computer companies, and by local industry which sees it as forcing up its own costs by making it buy higher-priced lower-quality Brazilian equipment.

Zagottis, a professor of engineering at the University of São Paulo, entertains no doubts about the effectiveness of the informatics policy. Brazil began at the same level as Mexico, but Brazil now has an industry of 60,000 employees, twenty times larger than Mexico's, and supplying over three-quarters of the nation's computers. "Informatics is connected to so many other fields, it is a strategic economic area which must be protected", he says. Protection is intended to come to an end in the early 1990s, to ensure that an inefficient monopoly does not develop.

Zagottis does not think the same policy will work in other high-technology areas. "You cannot have a market reserve policy for biotechnology", he says, "because you cannot do reverse engineering in biotechnology." In other words, while it may be possible to clone an IBM by 'reverse engineering' (IBM engineers might call it 'copying') it is not possible to clone a genetically engineered drug.

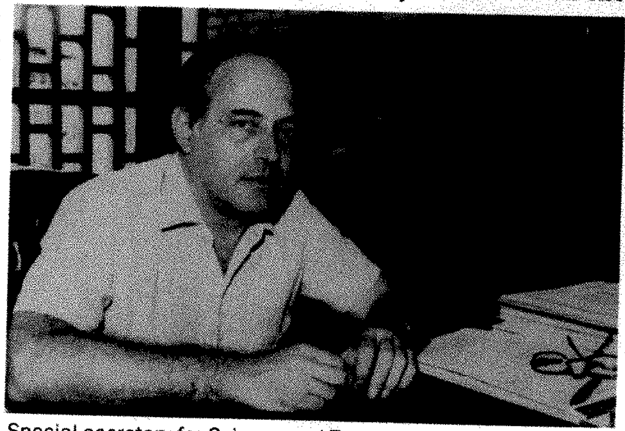
The immediate threat to the informatics law was nullified by the revival of the ministry, but the damage to the science and technology budgets could not be so easily repaired. For almost three months of turmoil, there had been no one to fight for science and technology. Some research institutes, such as the National Institute for Amazonian Research (INPA), that are directly attached to the ministry saw their budget appear in one agency, disappear, appear in another and then disappear again. The result is that they still do not have proper budgets for 1989. Massive cuts for the National Research Council, CNPq, and for the project research agency, FINEP, cannot now be fully restored.

Although it is too early to say what may happen after a new President of Brazil takes office early next year, Zagottis expects that the government will have no long-term investment capacity in the next few years. His solution is to turn to the World Bank and the InterAmerican Development Bank (IDB) to tide Brazil's scientific enterprise over the next next few years. Two big loans are under negotiation: one is a World Bank loan of $330 million to continue the Programme of Support for Scientific and Technological Development (PADCT), the other an IDB loan of $1,000 million over five years to support an integrated science and technology plan.

Zagottis says the idea is to use the money to "allow construction of new buildings for research centres, new laboratories and equipment and to train researchers and technicians". The World Bank is, however, not especially pleased with the handling of funds loaned to Brazil for PADCT I, six years ago. An official team sent to examine progress reported at the end of 1988 that if difficulties delaying the projects "are not solved urgently, they may also create serious difficulties in the renewal of the PADCT loans". Sluggish administration, "excessive" and "unjustified" delay in importation procedures, and the "enormous devaluation of allocated resources due to the current inflation rate if the money cannot be spent very quickly" were the principal complaints.

The importation delay for foreign equipment and materials is a terrible problem. Four-year delays are not uncommon. Zagottis claims that a new law will soon complete changes already under way and ease the import problem. Responsiblity for import procedures is being devolved to big universities from CNPq, which was exceedingly slow in handling requests. But there are, as Zagottis says, "problems lower down the hierarchy"; the requests must still pass through CACEX, the import control agency, whose bureaucrats mete out the same treatment on imports worth $100 or $1 million and insist on evidence that there is no Brazilian substitute.

But imports are just an irritant compared with the fundamental problem of lack of investment. Brazil spends only 0.7 per cent of GNP — a sum of $2,500 million — on science and technology, as against the 2.5–3 per cent spent by the industrialized countries ($150 million in the United States). Zagottis points out that 80 per cent of the money spent by Brazil on science and technology comes from the government, a far higher percentage than elsewhere. If Brazil can boost industry's contribution, then 3 per cent of GNP might be possible for Brazil too. Zagottis backs a short-term rise to 1 per cent of GNP by the government, followed by a steady series of small rises, always a little above the rise in GNP. The first goal, he says, is 2 per cent by 2000.


São Paulo

Special secretary for Science and Technology Zagoittis supports reserving the Brazilian market for nationally produced computers.

For this to happen, a national plan is needed as well as political stability, not something Brazil seems set to enjoy. Increases in investment by industry will only come from a well thought out programme of tax incentives. At present, it is more profitable for industry to license foreign technology than develop their own But a failure to act could jeopardize what has been achieved so far. A key strategy has been to send people abroad for advanced training — that is where a large part of CNPq's money goes. As Zagottis says, "There are 7,000 students taking PhD courses outside Brazil; if they don't have a place to come back to they will leave Brazil permanently" and the effort will have been wasted. ☐

GRANTS AND SCHOLARSHIPS

Finance from start to finish

Rio de Janeiro

FINEP, the Agency for Financing Studies and Projects, is an unconventional mix of a conventional research foundation and a conventional development bank. In principle, this gives it a unique power to take a project from the basic research stage, when a government grant is required, through to the development stage when venture capital or low-interest credit becomes important. In practice, things do not work out quite so neatly: FINEP finances a heterogeneous brew of projects, all at different stages, ranging from university research on AIDS to an industrial company's attempt to develop the first fully Brazilian automobile.

FINEP is one of the few federal agencies to have avoided the move to Brasília, something which clearly pleases its staff. Its offices are in downtown Rio de Janeiro.

Support for basic research comes principally through FINEP's role as executor of the National Science and Technology Development Fund (FNDCT), worth $107 million in 1987. The fund provides for infrastructure, new buildings and new equipment. Significant extra help comes through $180 million of World Bank funds, most of it matched by Brazilian funds, in the Programme of Support for Scientific and Technnological Development (PADCT). Grants are given to support projects, to upgrade and build new facilities and to support university graduate courses. Support for individual researchers is left to the National Research Council, CNPq.

"Earthquakes and turmoil" is how 1989 was described at FINEP. Thanks to the savage cuts of the Summer plan and the temporary disappearance of the Ministry of Science and Technology, to which FINEP is answerable, the agency will be lucky to receive 60 per cent of its 1987 budget. The World Bank has also been frank in its criticism of the slow progress in giving out PADCT funds. Of the $180 million loaned to Brazil in 1983, less than half had been disbursed by 1989. Import regulations and excess bureaucracy — little of it of FINEP's making — have been the major problems.

FINEP claims a good track record. When it was set up in 1967 to develop Brazil's engineering industry, there were very few companies, foreign or Brazilian. According to João Luíz Coutinho de Faria, president of FINEP, "twenty-two years later we have 200 companies; most are Brazilian with an annual turnover of $1,450 million".

FINEP has 700 staff, more than 100 with doctoral or masters' degrees, and last year supported 1,840 projects split almost equally between scientific research and technological development.

Coutinho de Faria points out that demand for the technological component was more than $1,000 million last year. He hopes to see FINEP's funds return to their 1987 level of $300 million next year and climb quickly to the $800 million mark. □

CAPES

Supporting graduates by algorithm

Brasilia

Postgraduate programmes throughout Brazil are coordinated by a Ministry of Education agency called CAPES; not surprisingly, few know the agency by its lengthy title of "Coordinating Agency for Advanced Training of High-Level Personnel". CAPES gives grants and fellowships to 14,000 students at Brazilian universities and to 2,000 students in North America and Europe.

Overall, CAPES provides backing for 700 graduate courses; two-thirds of all the courses in Brazil. It also runs a programme to help build up institutions so that they can offer new courses.

The graduate-training system grew rapidly in the 1970s during the expansion of the university system. At that time, many young and inadequately-trained staff obtained jobs. Today, the boom is over but there are still many unproductive faculty and graduate courses are of very uneven quality. CAPES makes sure it helps the good courses but is sometimes criticized for not being tough enough on the bad.

CAPES' director, José Ubirajara Alves, says that graduate courses are now the "best-evaluated sector in the science and technology system". He is a rare type in Brasilia, describing himself as just a "technician" who has come up with an "algorithm" to ensure that money goes to the courses that deserve it. In fact, Alves is a very well-respected mathematics professor with plenty of experience of starting graduate courses from nothing. When he joined the university at Fortaleza in his home state of Ceará in 1965 he was the only person on the staff with a doctorate (from the University of California, Berkeley). He established its first graduate course — the first at a university north of Brasilia. Fortaleza now has twenty courses.

Alves' algorithm takes into account the standard of the course, the numbers and types of professors and students, and the area (some types of research are more expensive than others). When the calculation is complete the budget goes to the university to provide for a quota of students, selected by the university. □

Fast forward for grants

São Paulo

When scientists complain that the National Research Council (CNPq) is too slow, or too bureaucratic, or too opaque, or too arbitrary, they often end by saying, "Why can't it be like FAPESP?" FAPESP is São Paulo state's own research foundation, receiving by law 0.5 per cent of state income each year to spend on scientific research.

FAPESP is fast: it normally makes decisions on grants within ten weeks of application, less than half the time required by CNPq (or by US NSF).

To ensure openness, applicants are given as much information about the progress of their grants as needed, a job which keeps a couple of secretaries busy full time. And once a grant is given, FAPESP will help to compensate for changes in exchange rate that push up the cost of imported materials. The foundation runs on 50 staff, against CNPq's 1,000.

Alberto Carvalho da Silva, FAPESP's director, is modest about the foundation's reputation, pointing out that CNPq has a budget twelve times as big as FAPESP's $42 million, and has to deal with unexpected cuts and political uncertainties. But he is critical of CNPq's staff numbers, saying that there are so many employees that it has to create something for them to do. Carvalho da Silva has been a victim of the workings of bureaucracy himself; he was purged by the military from his university post in 1969.

FAPESP's operations are much the same as those of research councils everywhere. Support goes to scholarships (not more than 50 per cent of the total), research awards, a small number of large long-term projects (most recently, support for participation in BITNET), visiting professorships, publications, meetings and so on. One novel programme provides 500 scholarships a year to encourage students to move into research by providing support for a pre-graduation research project.

Expansion and change are on the way. The new state constitution increases FAPESP's budget to one per cent of state tax income and gives it new responsibility for the support of technological research. Until now, 90 per cent of the budget has gone to basic research at the universities. In the future there will be a new commitment to the private sector on training and pre-competitive research. "Thus far, we've been run by scientists", says Carvalho da Silva, "now we'll be looking for international advice. We will use our contacts with the United States and the United Kingdom to look at science parks and adapt their models to our realities". □

RESEARCH SUPPORT

The council has the last word

Brasilia

It is a mean trick to arrive at the headquarters of the National Research Council (CNPq) after several weeks of accumulating complaints from Brazilian scientists about their parent research organization. CNPq is too cumbersome, they say, it is supposed to handle import procedures but everything takes years, travel grants arrive after conferences are over, foreign collaborations founder in paperwork, and grants are spread too thin, or go to foreign scholarships so that nothing is left for those at home.

And yet more, CNPQ swallows you up complains one University of São Paulo researcher, “if you criticize them, they say ‘come and help us’, and you end up being involved in all those difficulties too”.

Guilherme Brandão, Special Assessor for International Cooperation at CNPq, is a pipe-smoking meditative type who only occasionally shows signs of mild irritation at some of the more unreasonable behaviour of scientists. CNPq is not, in any case, a complete master of its own destiny, but has to weather all kinds of political storms and bureacratic meddling.

Science lives in a difficult environment. “Science and technology are not thought of as a high priority”, says Brandâo, “we are pressured by so many other things we need to solve, we have immediate problems in health and nutrition and so on”. And because there is no tradition of research and development by industry (expenditure on research by industry is just 5 per cent of the total), science is totally dependent on the government. “When there is a government financial crisis, we suffer badly”, says Brandão. One of those crises arrived earlier this year when budgets were cut by half and, for a short while, it seemed that some 40 per cent of the staff of CNPq's research institutes might be without a job.

To summarize, CNPq is a research council just like others elsewhere, except that along with its grants programme it has eleven research institutes (see page 357) and a large scholarship programme. It has been very powerful, with full responsibility for science and technology policy. But that responsibilty went to the Ministry of Science and Technology (now the Special Secretariat for Science and Technology) in 1985. CNPq has never really relinquished its claim to the area and despite lacking an official mandate, continues work at its Centre for Policy Studies.

If scientists complain of tardiness, they are perhaps insensitive to CNPq's scale. Each year, 40,000–50,000 applications for grants come in to CNPq's offices (including travel grants and so on), along with over 100,000 applications for scholarships. Suitable reviewers have to be found for each application, CNPq can provide for a third of the applicants in each category.

CNPq employs 1,000 staff but even so Brandão says that sometimes “the amount of paperwork drives us crazy here”. The system is to be rationalised next year. Instead of four fixed decision periods a year, a system which can never generate a decision in less than 4–5 months, CNPq will deal on a once-a-year basis with some things (scholarships, for example) and continuously with others that need immediate decisions (such as travel grants). That change should do away with a substantial fraction of the complaints of CNPq's inflexibility.

CNPq's scholarship programme is big, too big says critics, who think more of the resources should be directed to ongoing research rather than training. Ten year ago there were just 5,000 research scholarships in total. Today there are 30,000 inside Brazil and 3,500 overseas, part of a definite policy that top priority should go to human resources. But the number abroad will lessen next year as people return and efforts are made to save foreign currency. Inside Brazil the number will increase to provide 44,500 places. A scholarship is not riches — a doctoral student receives around $600 a month — but thanks to “a very great joint victory of CNPq and CAPES”, the amounts are tied to staff salaries so providing compensation for inflation.

On the extraordinary difficulty of importing equipment and reagents for research, Brandão wearily says, “Yes, we understand the community's complaints. But sometimes they complain in the wrong place”. CNPq is caught up in a much vaster bureaucracy.

All imports must pass through CACEX, and CACEX is essentially an instrument of foreign policy. It is necessary to show that no similar Brazilian product exists but ‘similarity’ is often a “complicated business”, says Brandão. Next a pro-forma invoice is necessary, then “when you send this to CACEX they may have a problem with foreign currency and freeze everything”.

While the difficuties are undoubtedly real, some problems may remain at CNPq. According to the World Bank, FAPESP, the São Paulo-state research council (see opposite) takes from “six months to a maximum of one year to import equipment”, while CNPq takes “one and a half to two years”. And CNPq took “at least 45 days to log in importation requests” (it has since improved).

CNPq has to spend a lot of time fighting off arbitrary decisions from elsewhere. Brandão relates how in January the government suddenly decided that money sent abroad for training grants should be exchanged at the tourist rate rather than the official rate, immediately cutting the grants' value by more than half. In the ensuing havoc, many students decided not to take up their scholarships. The decision was reversed only a few weeks ago.

One complaint which Brandão clearly feels unfair is that Brazil is trying to shut out foreign researchers or limit their access to the Amazon. Brandão says that 99 per cent of foreign expeditions (of which there are 30–40 a year) are approved, but there have to be formalities to ensure that an expedition will not intrude on Indian or private land and so on. Collaborative programmes, of which there are thousands, are organized through Brazilian counterparts. Brandão says the system ensures that Brazil obtains some benefit from research conducted here.

CNPq is looking to the future and looking for innovative ways to boost research investment by industry. It is not easy because in many industrial sectors a foreign licensing agreement captures a sure product while home development involves risk. But in other, newer sectors, foreign companies do not want to license their technology. CNPq is trying to get Congress to adopt flexible tax incentive strategies. In the meantime it is introducing a new programme of human resources in strategic areas (biotechnology, new materials and the like), to support the training of personnel in any institution, private or public, provided it is tied to a long-term research and development programme. The goal is to have 3,500 people in the programme by next year. □

CAPITAL CITY

Back to the future

Brasilia, the futuristic city of the 1950s, is laid out in the shape of a gigantic bow with its central arrow an enormous, thousand-yard wide avenue that, at its tip, juts out into an artifical lake. Along it, at great intervals, are the television tower, various monuments, the cathedral, the double row of identical, rectangular blocks containing the ministries, and then Congress and the Supreme Court.

The bow is a curving set of parallel roads along which are organized the city's ‘sectors’: the hotel sector, the banking sector, the cultural sector, the hospital sector, the embassy sector, the sector of individual housing . . . and so on. The great unifying architectural plan continues to inspire in areas of astonishing simplicity of design, where traffic flows swiftly past rows of simple, white concrete apartment blocks raised on stilts to allow clear sight lines. Elsewhere, the road plan remains but the city has filled in a medley of styles. CNPq has its offices in the sector of “autarquias” — autonomous organizations. □

BOMBS AND BIOTECHNOLOGY

Some projects from the past

São Paulo

WHEN the first wave of oil price surges arrived in 1973, Brazil reacted with two new and massively expensive programmes to reduce dependence on energy imports One was intended to provide electricity from nuclear power, the other to substitute sugar alcohol for petroleum Both projects were creations of a military government which could exercise strong central control over resources Fifteen years later, both projects are floundering as they face markets which demand a more rational allocation of resources

Opposition to the nuclear programme was strong from the start "It was very clear that nuclear energy for electricity production was a complete misconception," says University of São Paulo (USP) rector, José Goldemberg, a well-known critic of Brazil's nuclear plans, "seven billion dollars gone with little to show"

A small US reactor is in operation but the agreement signed with West Germany to build eight reactors has yet to create one operating plant The programme, "was slowly delayed but never cancelled," says Goldemberg, "scientists opposed it because it involved very little technology transfer Industrialists who lost opportunities because of support for the programme opposed it " Last week, West Germany extended the agreement with much reduced goals But the situation may change again under a new president Goldemberg believes that "in a democratic society it will become increasingly difficult to justify the expenditure against social programmes" The sugar alcohol programme appears very much more successful

Four million of Brazil's 12 5 million vehicles run on alcohol produced from fermentation of sugar and massive reductions in petroleum imports have been achieved But the programme is in serious economic and political trouble Brazil has found large oil reserves of its own Sugar alcohol is proving more expensive to produce than petroleum, the distilleries are producing huge quantities of polluted water, and subsidies for sugar-cane growing distort local economies by encouraging farmers to grow cane even when vegetables are in short supply

These, at least are the arguments advanced by the state petroleum industry, which wants to see petrol roll back some of alcohol's advance With the backing of the Ministry of Mines and Energy, the petroleum industry has scored well in the media war The sugar alcohol industry, backed by the Ministry of Industry and Commerce, is in retreat but it too claims that subsidies disguise the true state of the petroleum industry The battle of the subsidies will be fought between the two ministries under the new president and in the minds of consumers, who must decide which kind of car to buy

One factor in the battle is the scope for improvement in sugar alcohol production Waste treatment is already possible and biotechnology may offer much more Little sustained effort has gone into the improvement of yeasts Genetic engineering can permit new substrates to be used, such as starch which yeast cannot normally degrade In a clever piece of engineering, Ana Clara Schenberg at USP has already constructed yeast strains carrying α-amylase genes from mouse, plus other yeast genes introduced by conventional genetics and shown the new strain can produce ethanol directly from starch While the project is at the laboratory stage, it suggests that it is too soon to begin writing off sugar alcohol ☐

Parallel secrets

FEARS that Brazil is intending to develop nuclear weapons in secret are fading with the return of democracy

In 1987, after the disppearance of the military regime, a secret 'parallel' nuclear programme emerged with the announcement by President José Sarney that the navy had succeeded in low-level enrichment of uranium fuel The work was carried out at the Institute for Energy and Nuclear Research (IPEN), adjacent to the USP campus

The aim of the programme is not a bomb, but fuel for a nuclear-powered submarine Although Brazil has no enemies and the expense of developing nuclear submarines seems superfluous, the offical reply is that the project will take 20 years and by then Brazil will be a great power The navy was much impressed by the performance of nuclear submarines during the Falklands conflict

As the 'parallel' programme went public, demands increased for greater accountability of the military's activities The new constitution prohibits the development of nuclear weapons and gives Congress control over the nuclear energy programme But the constitution left possible the development of 'peaceful' nuclear explosives The first steps towards civilian control came last year when the government established a Superior Council for Nuclear Energy as an advisory board to the president Goldemberg was one of three civilian scientists on the council, which includes 17 ministers Although the meetings are not frequent, Goldemberg says that "the president attends and it is now very difficult to do anything under cover"

Next step could be a congressional committee with oversight of military programmes Brazil would then be close to the certainty that the submarine programme could not be diverted towards manufacture of a bomb ☐

. . . and future

Rio de Janeiro

IF new styles of collaboration between the universities and industry are what is needed to boost investment in science, then the Federal University of Rio de Janeiro (UFRJ)'s island may be the place to look for future trends Here Antonio Paes de Carvalho has confidently launched a new biotechnology venture, Bio-Rio, which links industry, the Oswaldo Cruz Foundation and the university Within ten years, Paes de Carvalho confidently predicts there will be 70 companies here, employing some 4,500 people and enjoying $250 million in sales

Paes de Carvalho is a cardiac electrophysiologist, an unusual skill from which to move into biotechnology But in 1980, he says, he was director of the Institute of Biophysics at UFRJ and became "worried about the financing of research" As there were experts in tissue culture and molecular biology in the institute, Paes de Carvalho thought biotechnology might be a good bet

So with the belief that "you have to do things yourself", he set up a non-governmental, biotechnology foundation close by. The foundation will operate central research facilities and allocate surrounding land, at a nominal cost, to private enterprise The central facility will offer specialized services (for example, peptide and nucleotide sequencing and synthesis) and provide 'incubator' space where projects can be developed

In October the central laboratory facility was being carved out of the vast kitchen (conveniently complete with cold storage rooms) of an abandoned university cafeteria This month, the first two companies are moving into their incubator space

There are other biotechnology intiatives elsewhere but none based on direct interaction between industry and university "The biggest fight," says Paes de Carvalho, "is to convince the university that this kind of industrial proximity is OK'. The university authorities at first rejected the project, but were eventually persuaded with help from the rector — as he was a communist he apparently could not be accused of selling out to capitalists

The government has invested $3 million in Bio-Rio, and industry another $1 million Paes de Carvalho expects industry to provide another $100 million over five years, encouraged by big incentives from the National Development Bank Critics say the plan's weakness is the shortage of qualified researchers, most of then in universities and without interest in links to industry

But Paes de Carvalho believes that he can engineer the interaction between university and industry so that it will be painless for researchers and not threaten basic research ☐

An Invitation to Explore the Frontiers of Knowledge

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- *Towards a Quantitative Theory of Intellectual Discovery*, R.G. Fowler, University of Oklahoma.
- *The Nature of Time*, Y. Terzian, Cornell University.
- *Engineering Anomalies Research*, R.G. Jahn, B.J. Dunne, and R.D. Nelson, Princeton University.
- *The Strange Properties of Psychokinesis*, H.Schmidt, San Antonio.
- *Archeological Anomalies in the Bahamas*, D.G. Richards, Atlantic University
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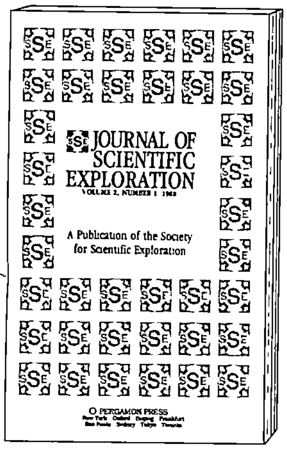

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Upper bounds on 'cold fusion' in electrolytic cells

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Experiments using three different calorimeter designs and high-efficiency neutron and γ-ray detection on a wide range of materials fail to sustain the recent claims of cold fusion made by Fleischmann *et al*[1] and Jones *et al*[2]. Spurious effects which, undetected, could have led to claims of cold fusion, include noise from neutron counters, cosmic-ray background variations, calibration errors in simple calorimeters and variable electrolytic enrichment of tritium.

RECENT publications[1,2] reporting electrochemically induced nuclear fusion, at room temperature, have aroused great interest. The signatures reported are excess heat output, neutron emission and tritium generation from cells with palladium cathodes[1], and neutron emission alone at a much lower level from cells with titanium cathodes[2]. Conventional nuclear physics predicts that fusion between light nuclei requires either very high temperature (as in a tokamak) or unusually close proximity of the two nuclei (as in muon-catalysed fusion). The calculated fusion rate at the internuclear separation in the deuterium molecule (0.74 Å) is $\sim 3\times10^{-64}\,s^{-1}$ (ref 3), so the rates reported in ref 2 ($\sim10^{-23}$ d-d-pair^{-1} s^{-1}, d is deuteron) are not easy to understand in the context of the known interstitial-site separations (~3 Å), even more so when it is realized that this rate is a severe underestimate because of incomplete deuterium loading in titanium (see Materials characterization section). The reaction rates reported in ref 1 are even more difficult to understand[4], and the existence, despite strong arguments to the contrary (ref 5, for example), of an unknown mechanism, which results both in an extraordinary enhancement of the reaction rate and a suppression of the normal nuclear-reaction channels, has been postulated.

In the first reports of the electrolytic cold fusion effect it was stated that the effect is not consistently reproducible, and that it both takes some time to appear and that it may subsequently disappear. Because electrochemical phenomena can be sensitively affected by the state of the surface, some irreproducibility is not, in itself, surprising, and other recent reports (refs 6-8, for example) give well documented accounts of failures as well as successes (R. A. Huggins and A. J. Appleby, Workshop on Cold Fusion, Santa Fe, May 1989) in observing the claimed effects. The clear lack of reproducibility necessitates significant replication, with controls at least equal in number to the number of tests, if positive results are to be viewed with confidence, and exploration of many different, well characterized, material and electrolyte combinations. Particularly, the timescales and achievable concentrations for electrolytic loading of deuterium into palladium and titanium, the quantities of hydrogen 'impurity', and the species detectable by surface analysis of used cathodes ought to be determined.

Calorimetry

We used three types of calorimeter. First, we built calorimeters of size and design similar to those used by Fleischmann *et al* (ref 1 and M. Fleischmann, personal communication) (Fig. 1). We found these to be inaccurate instruments with some very subtle sources of error which it is necessary to appreciate and analyse in detail. We used sixteen such cells, containing different-size cathodes (1-, 2-, 4- or 6-mm Pd rods) and different electrolytes (0.1 M LiOD, 0.1 M LiOH, 0.1 M NaOD or 0.1 M NaOH). Figure 2*a*,*b* shows the results obtained for a typical cell. An immediately evident characteristic is the sloping baseline, with the sawtooth pattern a consequence of the regular refilling of the cell. The baseline slope can be quantitatively accounted for by a variation of the calibration constant, *k*, with the level of liquid in the cell. This is a result of radiative losses through the vacuum jacket[9] and conduction up the glass inner wall of the cell[10] (see Fig. 2 legend). A consequence of the sloping baseline is that any calibration is only valid for a particular liquid level. We regularly refilled these cells to a reference level at which the calibration had been performed. We calibrated the cells with the heaters before the electrolysis was started and again much later in the run, during the electrolysis, as is evident in Fig. 2*a* (see Fig. 1 legend for details of the calibration procedure). There was no statistically significant difference between the different calibration sets, so all data were combined.

Figure 2*c* shows results for two of the calorimeters at the calibrated liquid level, in the form of percentage excess of apparent output power over the Joule input power. There was apparently an endothermic period at the beginning of the run, whose duration increased with cathode diameter. We speculate that this was due in part to poor stirring of the solution during hydrogen uptake by the cathode which caused a temperature gradient in the cell (gas is not at first evolved at the cathode and the larger diameter cathodes were shorter, to keep a constant surface area). An analogous effect at the start of the electrolysis was reported by Lewis *et al*[8], who showed that the apparent heating coefficient of a similar cell varied during the first part of a run. Because of this effect, we excluded the first 10,000 minutes of data from each cell when calculating the statistics. Table 1*a* shows the mean absolute power deviation for each cell and the standard deviation in this value. The standard deviation for all of the H_2O cells was not significantly different from that of the D_2O cells (*F* test, 1% level) so that the data from all of the cells can be used to estimate the error $\sigma = 0.048$ W (that is ±5-10%). A more detailed analysis of this error[10] showed it to be largely determined by the variability in the liquid level after refilling the cell, but also with a contribution from unquantified variations in the heat loss by conduction up the calorimeter wall to the air above the water bath. Compared with these errors, effects that resulted from temperature gradients inside the cells were minor. The design used here varied from that in ref 1 in that the glass sleeve that supported the calorimeter and which was in direct contact with the inner wall, provided a large area of thermal contact with the water bath, and thus reduced the effect of the ambient temperature variations.

As we expected, occasional points from both H_2O and D_2O cells lay outside the 'control limits' of $\pm2\sigma$ (Fig. 2*c*). No points lay outside $\pm3\sigma$. No cell showed two or more consecutive points outside $\pm2\sigma$, and the number of points lying above the control limit in the D_2O-cell experiments was no different from that in the H_2O-cell experiments Therefore, we conclude that, within the experimental error, there was no significant anomaly in the behaviour of the D_2O cells compared with that of the H_2O cells If, however, the mean power deviation (Table 1*a*) for all eight of the D_2O cells (+9 91 ± 0 47% or 0 021 ± 0 004 W, mean Joule input 0 96 W) is tested against that for all eight of the H_2O cells (−2 46 ± 0 72% or −0 018 ± 0 004 W, mean Joule input 0 81 W for LiOH cells and 0 61 W for NaOH cells), it is clearly highly significant Because no sequence of individual points lay outside the control limits established above, we suspect that there is another, unknown source of error that scales with the input power This, of course, could be the postulated 'fusion effect', but the magnitude of the effect is commensurate with the errors The only reliable way of checking this is to construct calorimeters that are free of major sources of errors, and in particular, do not have a sloping baseline We therefore also used both isothermal and steady state (heat flow) calorimeters which satisfy this criterion

We have also analysed in detail the slope of the output curve to look for momentary power pulses on timescales shorter than the interval between calibrated points If the Joule input remains constant then this slope should not vary Any sudden or momentary extra power input, q_c, would change the slope by approximately $(k_{sb}+k_{c,0})q_c/M_0$ (M_0 is the water equivalent of the calorimeter, other symbols defined in Fig 2 legend) The number of significant deviations from the mean slope was found to be roughly the same for both the H_2O and the D_2O cells For the whole data set, the largest power excursion for a D_2O cell (6-mm

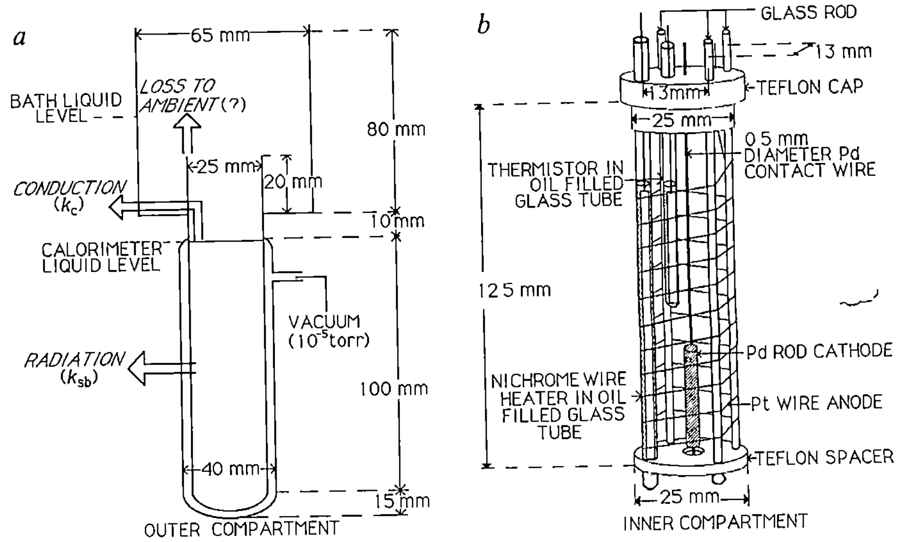

FIG 1 Schematic diagram of the FPH-type heat-flow calorimeter used here Heat flow paths are indicated here and discussed further in Fig 2 legend

METHODS As well as the Fleischmann, Pons and Hawkins (FPH) type, we used[10] two other calorimeter types—an improved heat-flow calorimeter (IHF) and an isothermal calorimeter The IHF calorimeters differed from the FPH type in three important ways First, they were larger a 500-ml-capacity cylindrical vessel constituted the cell, with anode-cathode spacing ~2 cm Second, the electrolysis vessel was inserted, using a film of oil for thermal contact, into a tightly fitting aluminium can which was itself packed around with insulating material and placed in a Dewar flask Whereas the temperature of the cell contents in the FPH calorimeter was determined using a glass-clad thermistor immersed in the cell itself, in the IHF design the temperature of the aluminium can was measured the can defined an isothermal surface for conduction of heat away from the cell and its use as the temperature measurement surface eliminated the sloping-baseline problem of the FPH design Third, the space within the Dewar flask above the electrolysis vessel was filled with a polystyrene cap extending well above the Dewar flask, with the aim of significantly reducing unquantified heat losses to the atmosphere The two types of heat-flow calorimeter were operated in water baths held at a constant temperature of 20 °C (±0 08 °C) The tops of the water baths were covered with a polystyrene lid In both cases the cells comprised a spirally wound Pt wire anode (0 25 mm) and a central Pd cathode (Johnson Matthey) For the FPH cells we prepared the different electrolytes using either conductivity water (H_2O cells) or slightly tritiated D_2O (specific activity 13 kBq ml^{-1}) of isotopic purity initially >99 9% We used 0 1M LiOD (D_2O from Aldrich, measured >99 9% initial isotopic purity) as the electrolyte in the IHF calorimeters The Pt and Pd contact wire was shrouded in glass tubing in the IHF cells to prevent any possible catalytic recombination of the electrolysis products In later experiments with the FPH cells we also used screened electrode contacts, but this had no effect on the results obtained The IHF cells contained a Pd wire electrode insulated to the very tip, which was positioned about three-quarters of the way up the cell This was used to define the internal liquid level during filling, or refilling, of the cell The larger volume of electrolyte in the IHF cells (300 ml) meant that refilling was required only occasionally, but the large thermal mass meant that the response was slow (time constant ~12 h) thus they were not sensitive to small bursts of heat We calibrated the heat-flow calorimeters using a nichrome wire heater placed in an oil-filled glass tube which was in contact with the cell contents The calibration procedure involved operating the heater, either without electrolytic current, or with a current significantly less (0 2–0 4 times) than the normal electrolysis current, until a steady-state temperature was attained With the FPH cells, use of a lower electrolysis current ensured continued stirring while diminishing the errors arising from the baseline drift, before each individual calibration the cells were refilled An empirical calibration curve was thus obtained by fitting the observed thermistor resistance, *R*, to a range of applied powers, *P*, using the equation $P=a-b(\log R)+c(\log R)^2$, where *a*, *b* and *c* are the fitted parameters The standard error of estimate on the fitted curve varied from 10 to as much as 70 mW with the FPH cells, largely reflecting the error in extrapolation to the reference level Neutron counting was performed in conjunction with the operation of the FPH cells, using two independent banks of counters mounted above the cells, on top of the water-bath cover[10] Detection sensitivity above background was 3 event s^{-1} in the cell We never observed a signal above background The isothermal calorimeter (J A Mason, R W Wilde, J C Vickery, B W Hooton and G M Wells, Proc 29th A Meet Inst Nuclear Materials Management, Las Vegas, Nevada, June 1988) comprises three concentric aluminium cylinders each separated by a heat transfer medium with a relatively low thermal conductivity The cylinder temperatures are maintained by electrical heaters wound as helical coils around each cylinder Temperature control is achieved by resistance thermometers on each cylinder which are used in conjunction with classical control software and the cylinder heaters The rate of thermal energy evolution in the measurement chamber (12 5-cm diameter, 26 cm high) is determined by measuring precisely the electrical power supplied to the chamber We operated the calorimeter at a measurement-chamber temperature of 42 ± 0 001 °C The measurement-chamber power resolution is <5 mW for an operating power of 20 W The electrolytic cell contained ~1 l of 0 1M LiOD The cathode was contained in a perforated glass canopy to prevent the evolved gases from mixing inside the measurement chamber The anode was a Pt foil cylinder 3 cm high and 12 cm in diameter surrounding the cathode The cell was thermally coupled to the calorimeter measurement chamber by conducting oil Measurements using Pt cathodes and also using nichrome wire heaters showed that there was a small systematic error in the calorimeter, an apparent power excess that varied linearly with the input power up to 100 mW for an input of 15 W A linear fit to these measurements (12 points, standard error of estimate 8 8 mW) was therefore used to apply a correction to the apparent excess measured for the Pd cathodes The measured output power of both the isothermal and IHF cells was corrected for the power loss that is due to evaporation of the electrolyte, assuming that the electrolysis gases were saturated in water vapour as they passed out of the cell (25 0 mW A^{-1} at 42 °C) $q_v=(p_v/p_0)(1\ 5)\Delta H_v I/(2F)$ where q_v is the power loss, p_v denotes the saturation vapour pressure and ΔH_v the latent heat of evaporation of the electrolyte, p_0 is the atmospheric pressure *F* is the Faraday constant and *I* is the current (assumes ideal gases)

TABLE 1 Calorimetry results

a) FPH Calorimeters current 300 mA, current density 80–110 mA cm^{-2}, 0.1 M LiOH, NaOH, LiOD, NaOD

Pd[a]			H_2O Cells		D_2O Cells[b]	
Diameter Surface area	Volume Charging time	Cation	Mean Joule input[c] (mW)	Excess[de] (mW)	Mean Joule input[c] (mW)	Excess[de] (mW)
6 mm 3.9 cm^2	0.28 cm^3 870 h	Li	820	−45±50	960	12±57
		Na	590	14±71	980	57±44
4 mm 3.5 cm^2	0.19 cm^3 810 h	Li	800	−19±28	960	−20±29
		Na	610	−55±53	960	−11±49
2 mm 3.1 cm^2	0.094 cm^3 740 h	Li	780	40±53	930	61±26
		Na	620	−12±38	940	36±40
1 mm 2.8 cm^2	0.071 cm^3 670 h	Li	830	−46±64	980	43±64
		Na	620	−18±60	980	−4±35
Mean excess	$\pm(\sigma/\sqrt{n})$ (mW)[f]			−18±4 (n=143)		+21±4 (n=146)

b) IHF and isothermal calorimeters 0.1M LiOD

	Pd					
Calorimeter type	Type Surface area	Total charging time Volume	Current (mA cm^{-2})	Time (h)	Joule input[c] (W)	Excess[d] (mW)
IHF	2-mm rod[a] 3.1 cm^2	797 h[l] 0.16 cm^3	156	231	2.341	78±77[m]
			156	206	2.380	27±48
			219	187	3.842	−1±58
			279	173	5.188	−55±101
	Cast[g] 14 cm^2	797 h[l] 0.88 cm^3	35	236	2.172	100±40
			36	201	2.219	47±36
			49	187	3.489	−31±51
			62	173	4.863	−120±86
Isothermal	2-mm rod[a] 1.3 cm^2	284 h[b] 0.063 cm^3	159	284	0.925[o]	−10±12[n]
	Cast beads[h] 5 cm^2	355 h[b] 0.5 cm^3	50	7	0.985	33±12
			30	15	0.415	11±11
			70	7.8	1.796	5±12
			20	15	0.214	10±11
			100	8.6	3.405	−5±12
			120	270	4.825	2±13
			40	8.1	0.675	7±12
			80	5.7	2.311	9±12
			200	4.8	12.314	−10±12
			160	4.1	8.140	−17±12
			220	4.8	14.675	−9±12
	Melt-spun-ribbon[i] 74 cm^2	74 h[l] 0.35 cm^3	14	74	8.991	64±17
	2-mm rod[j] 1.3 cm^2	323 h[l] 0.066 cm^3	152	70	1.091	7±11
			530	253	10.358	15±14
	8-mm bar[k] 28 cm^2	520 h[l] 1.5 cm^3	30	520	8.974	36±15

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[a] Johnson Matthey (JM) 'Specpure', drawn from sintered stock prepared from high-purity powder.

[b] D_2O from Harwell reference stock, contains 13 kBq ml^{-1} tritium.

[c] Mean Joule input power supplied to cell (see Fig. 2 legend). Values for IHF and isothermal calorimeters have been corrected for heat loss that is due to evaporation (see Fig. 1 legend).

[d] Excess power = measured cell output power − calculated Joule input power.

[e] (Mean ± 1σ) of values calculated after each refilling to the reference level, excluding first 10,000 minutes of polarization (see text).

[f] Mean and standard deviation of the mean calculated for all H_2O data points and all D_2O data points.

[g] Specially produced material supplied by JM—prepared from cast Pd stock that was argon-arc melted into rod form using a gravity casting process. The rods were subsequently sliced and bent to decrease the loading time required (maximum distance from bulk to surface ~1 mm). The sample was cleaned using acetone, 10% HCl and distilled water.

[h] 'Specpure' Pd arc melted three times under argon on a water-cooled copper hearth.

[i] A variety of ribbons prepared (JM) by melt spinning of cast or sintered Pd. A proportion of the ribbons were heat treated (JM) for 20 min at 100 °C under 10% H_2/N_2. Ribbon thickness, 125 μm.

[j] Type [a] that was subsequently vacuum degassed at 1,200 °C, and loaded with deuterium at a pressure of 40 bar. The sample was cooled to liquid-nitrogen temperature before transferring to the calorimeter to minimize loss of D_2.

[k] Sintered high-purity bar, sliced and bent to decrease the loading time required (maximum distance from surface to bulk ~1 mm).

[l] D_2O from Aldrich Chemical Co., contains ~15 kBq ml^{-1} tritium.

[m] Mean and standard deviation of all data points after temperature stabilization; data point every 3 min.

[n] Standard deviation given by $\sigma=\sqrt{\sigma_b^2+\sigma_y^2+\sigma_c^2}$ where σ_b is the standard deviation of the baseline measurement, σ_y that of the power measurement with the cell running and σ_c is the standard error of estimate of the correction line (see Fig. 1 legend). σ_b and σ_y were typically 6 mW, σ_c was 8.8 mW.

[o] Small error in this particular measurement gave rise to the apparent small endotherm.

rod) was ~45 mW and for an H_2O cell (6-mm rod) ~40 mW These are small compared with the input power It is certainly clear that no unusually large power pulses occurred Given the difficulties in operating these calorimeters, very occasional occurrences of small fluctuations cannot be considered as support for a 'fusion' hypothesis

The other two types of calorimeters used in this study are described in Fig 1 legend and results are given in Table 1*b* We explored a range of different preparations of palladium and of current density, up to nearly 600 mA cm^{-2} With the improved heat-flow calorimeters (IHF), the sign and magnitude of the power excess varied with Joule input power, but was always <5% Expressed in terms of the volume of the palladium cathode, this sets a limit of 100–500 mW cm^{-3} The most accurate calorimeter used was the isothermal calorimeter (minimum-detectable power change ~10 mW, minimum-detectable energy in any brief burst ~40 J) We analysed these results at four-hour intervals by averaging both the Joule input power and the measured output power over a period of ~20 min Inspection of the data collected every four minutes between the regions of analysis showed no obvious signs of any short heat 'bursts' and we found no trend with time of the measured output power under any of the conditions used Table 1*b* shows that we obtained thermal balance to better than 20 mW (24–240 mW cm^{-3} Pd) We observed slight thermal excesses (30–60 mW) during the initial charging period of the palladium beads, and during runs with high-surface-area cathodes at high current it can reasonably be assumed that a small amount of recombination (4% at most) was responsible for this effect

Neutron counting

We investigated the emission of neutrons from a wide range of cells using three different detector systems (Table 2) The large, high-efficiency detector with which most of the neutron measurements were made is an oil-moderated assembly of 56 $^{10}BF_3$ proportional counters configured as 5 concentric rings[11,12] The total efficiency for 2 45-MeV d–d neutrons is 44% We built an automatic cell shuttle mechanism to exchange regularly two nominally identical cells, only one of which was powered This enabled the background (which is due mostly to cosmic rays, there being no anti-coincidence counter arrangements) and any signal to be counted virtually simultaneously In operation the cells were exchanged every 5 min, and the data from the 5 rings were recorded separately Data from a typical run are shown in Fig 3 as differences in the count rate between the powered and unpowered cell Although in the particular example shown, two spikes can be seen in the count rate differences from the detector as a whole, it is clear that these spikes are due entirely to the misbehaviour of ring 4, and are therefore spurious

The details of the cells and results of the measurements are given in Table 2 The lowest limits (2σ) on neutron emission derivable from Table 2 for palladium are $1\ 5\times10^{-2}$ n s^{-1} g^{-1} or $1\ 5\times10^{-2}$ n s^{-1} cm^{-2}, if the emission were sustained over the whole run For titanium, the values are 3×10^{-3} n s^{-1} g^{-1} and 4×10^{-3} n s^{-1} cm^{-2}, although the latter limit is too high because it does not apply to the runs using granules having a large and indeterminate surface area If we assume the emission to be sustained over only a one-hour period at most, then the limits are, for palladium 7×10^{-2} n s^{-1} g^{-1} or 4×10^{-2} n s^{-1} cm^{-2}, and for titanium 8×10^{-3} n s^{-1} g^{-1} or 2×10^{-2} n s^{-1} cm^{-2} (much less in the runs with granules) The neutron-emission rate limits are several orders of magnitude below the rates of ~10^4 s^{-1} reported in ref 1 and about one order of magnitude below the rate of ~0 4 s^{-1} reported in ref 2 It is significant that the limits in our work were also obtained for cells in which cold fusion could be expected to be enhanced, in particular by using titanium cathodes in the form of a large (40 g) mass of porous granules to increase both the reaction volume and the surface area, and an electrolyte specifically chosen to promote deuterium loading into the cathode[13] (see Fig 3) As discussed later, we have not expressed the results in terms of the number of deuterium pai within metal lattices

Because the net count rates given in Table 2 are differenc between much larger background rates, runs were undertak with a calibrated ^{252}Cf source (0 024 ± 0 003 fissions s^{-1}) emitti 0 09 ± 0 01 n s^{-1} and a physically identical blank The result wa 0 14 ± 0 05 n s^{-1}, in satisfactory agreement Furthermore, th expected neutron output of the unpowered UPt_3 cell, which due to spontaneous fission of ^{238}U, was 0 22 s^{-1} The valu measured in comparison with an empty cell was 0 20 ± 0 06 s Our confidence in the results of the shuttle differences seem justified Measurements on $CeAl_2$ and UPt_3 cells were include in the hope that high effective electron masses correspondin to these metal crystal lattices would mimic in some way th fusion enhancement effect of 'heavy electrons' such as muo in binding the deuterium nuclei closer together The palladiu ribbon run with the cell power switched on and off every tw hours (which was about eight times the characteristic diffusio time in the ribbon) was undertaken to enhance the appearan of non-equilibrium effects, and results (negative) are given Fig 4

γ-ray counting

As it has been shown that cold proton–deuteron (p–d) fusio is expected to proceed at rates greater by ~8 5 orders of magn tude than d–d fusion[3], we carried out an alternative investigatio of electrolytic enhancement of hydrogen-isotope fusion by look ing for the D(p, γ)^{3}He 5,488-keV γ-rays from p–d fusion W used a lead-shielded 113-cm^3 n-type high-purity germ[illegible] (HPGe) crystal γ-ray spectrometer to search for any γ-[illegible] the energy range 0 1–7 MeV emitted by a variety of cold [illegible] cells operating with a mixture of light and heavy water [illegible]h results, also given in Table 2, are consistent with no γ-ra emission The lowest limits (2σ) on γ-emission derivable from Table 2 are, for palladium, 7×10^{-3} γ s^{-1} g^{-1} or 1× 10^{-3} γ s^{-1} cm^{-2}, and for titanium $1\ 4\times10^{-3}$ γ s^{-1} g^{-1} or 4× 10^{-4} γ s^{-1} cm^{-2}, if we assume that the emission is sustained ove the whole run Jones *et al*[2] report that the fusion activity ma last for only 4–8 h and begin ~1 h after the cell is powered, an in these circumstances typical values for the standard deviatio in the γ-ray emission rates are ~0 05 s^{-1} and the 2σ limits fo titanium are 4×10^{-3} γ s^{-1} g^{-1} or 3×10^{-3} γ s^{-1} cm^{-2} Post-ru analysis of the hydrogen isotopes taken up by the palladiu cathodes with the mixed light and heavy-water electrolytes use gave very satisfactory D H ratios, lying in the range 1 5–2 5 We note that if the proposed enhancement of the fusion proces is as valid for p–d as for d–d fusion then, because of the enhanc[illegible] tunnelling in the lighter d–p system[3], the γ-ray measurement actually provide a much more stringent limit on the cold fusio process than do the neutron measurements The interpretatio of the results from some of these cells was complicated by th dissolution of gold anodes and the consequent gold depositio onto the cathode this also would have affected the origina work[2]

Tritium enrichment

Fleischmann *et al*[1] claimed a tritium production rate o ~10^4 atom s^{-1}, commensurate with their reported neutro emission rate They used a differential technique in which th tritium accumulation in a cell with a palladium cathode wa compared with that in a cell with a platinum cathode They too samples for analysis at regular intervals and maintained a constant total electrolyte volume by the addition of fresh D_2O

Electrolytic enrichment is widely used to increase the concentration of tritium in water before analysis[14] Reproducibl results require careful control of the electrolysis, as the enrichment factor can vary widely (refs 15, 16 and R L Otlet, persona communication) important effects are seen with change o electrode materials, with variation in the condition (activity) o the electrode surface, with the current density (overvoltage) an with the temperature Without precautions, the variations in enrichment factor can be more than a factor of two[16] The claims therefore need to be assessed against this known variable electrolytic enrichment Surprisingly, Fleischmann *et al*[1] claimed that there was no electrolytic enrichment in their platinum cell

If the rates of electrolytic evolution of hydrogen isotopes are written as follows

$$D_2O \rightarrow D_2 + \tfrac{1}{2}O_2 \qquad \text{rate} = r$$

$$DTO \rightarrow DT + \tfrac{1}{2}O_2 \qquad \text{rate} = XSr$$

where r denotes the electrolysis rate, S the tritium-deuterium separation factor and X the mole fraction of tritium, T, in the solution, then the tritium accumulation is given by[10]

$$\frac{X}{X_0} = \frac{1}{(S+\alpha+\gamma)}\left\{1+\alpha+\gamma-(1-S)\exp\left[-\frac{(S+\alpha+\gamma)rt}{N_0}\right]\right\} \qquad (1)$$

where the total solution volume is maintained constant by the addition of fresh D_2O (containing a mole fraction X_0 of tritium, directly proportional to the disintegration rate), the ratio of the sampling rate of the solution to the electrolysis rate is α and γ denotes the ratio of the rate of evaporation of the solution to the electrolysis rate—a small correction that may be calculated assuming that the electrolysis gases passing out of the cell are saturated with water vapour On the timescales of interest, variability in S would give a variable enrichment

$$\delta\left(\frac{X}{X_0}\right) = -\frac{rt}{N_0}\,\delta S \qquad (2)$$

If the solution becomes contaminated by hydrogen absorption from the atmosphere, then if S_{obs} denotes the apparent DT enrichment factor derived from the application of equation (1), $S_{obs} = S/[0\ 5(1+f)]$, where $f = (1-X_H)/(1+X_H(S_{HD}-1))$ with X_H denoting the mole fraction of hydrogen and S_{HD} the HD separation factor (relative rate of reaction of HDO and D_2O) Therefore, as well as the inherent variability from one electrode to another, it is evident that any variability in the amount of hydrogen pickup will give a variation in the apparent enrichment factor

$$\frac{dS_{obs}}{dX_H} = \frac{S_{HD}}{2[1+X_H(S_{HD}-1)^2]}$$

We found that, unless exceptional precautions were taken (and we believe that we used experimental procedures very similar to those used in ref 1 (M Fleischmann, personal communication)), values of $X_H \approx 0\ 07$ were common Under these conditions, a change of X_H of only 0 01 would give a change in S_{obs} of 0 02, which could be significant given the smallness of the claimed effect.

Any assessment of whether differential enrichment can be considered to account for the results in ref 1 depends critically on the value of X_0, which was not reported Using values[1] of r ($1\ 24\times10^{18}$ atom s^{-1}) and N_0 ($14\ 6\times10^{23}$ atom) we calculate from equation (2) that if X_0 were at the extreme low end of the range for commercial D_2O (3 Bq ml^{-1}), a value of $\delta S = 0\ 46$ would be required, if it were moderate (10-15 Bq ml^{-1}) a value $\delta S \approx 0\ 1$ would be needed, if it were high (80 Bq ml^{-1}) $\delta S \approx 0\ 02$ would suffice

The applicability of equation (1) was confirmed experimentally, on both platinum and palladium cathodes, in conjunction with calorimetric and neutron-counting experiments (see Fig 5 legend) using D_2O with an initial tritium content of 13 kBq ml^{-1} (efficiency corrected) The fit of this equation to all the experimental data (Fig 5) gave, for palladium, $S_{obs} = 0\ 59$, and for platinum, $S_{obs} = 0\ 61$ Correction for the uptake of hydrogen, using $S_{HD} = 6$ (ref 16), gave $S \approx 0\ 48$, in agreement with previous work and theoretical expectations ($S = 0\ 46 \pm 0\ 02$, (refs 14, 15 and D S Rawson and R L Otlet, personal communication)) For individual electrodes, S_{obs} for palladium varied from 0 46 to 0 65 (corrected S from 0 42 to 0 58), whereas S_{obs} for platinum varied from 0 56 to 0 85 (corrected S from 0 43 to 0 69)—a total range for individual electrodes of $\delta S_{obs} = 0$ which is enough to account for the results of Fleischmann *et al*

It is clear from these results and discussion that more evidence needs to be presented before the tritium accumulation reported can be considered as experimentally reliable evidence for the occurrence of a fusion process

Materials characterization

With 125-μm palladium foils, hydrogen loadings (determined[1] by hot-extraction mass spectrometry of specimens frozen in liquid nitrogen, and independently by electrochemical extraction) of H/Pd = 0 95±0 05 were achieved in ~1 h a 100 mA cm^{-2} in 0 1 M LiOH solution with a platinum anode The limit of deuterium loading in 0 1 M LiOD was lower (0 84± 0 03) Rods (1- and 2-mm diameter) polarized for extended periods in neutron counting and calorimetry experiments showed D/Pd = 0 76±0 06 It is well known[17] that the equilibrium pressure for a given deuterium loading is higher than tha for the same hydrogen loading Current-interruption method confirmed that overpotentials in the range 0 8-1 V (ref 1) were being obtained From an initial composition >99 9% D_2O, the solutions degraded to ~98 5% in 24 h and to 88-98% (analysi by infrared spectrometry) following electrolysis for many weeks The resulting ratio H/D in the palladium was 0 02-0 04

During electrolysis, all of the palladium cathodes becar covered with a layer that varied in appearance from [illegible] tarnish to a dense jet black In the latter case, loose black materi was also formed, which in extreme cases came off during electrolysis, resulting in quite heavy erosion of the cathode The layer itself evidently represented a modification of the morphology of the cathode at the surface The formation of a thick black layer was enhanced at high current density, at high temperature, on smaller diameter wire and by frequent abrup alterations of the current density repeated over a long period The layer was more noticeable on the outside of a spiral-woun cathode than on the inside The layer was also more noticeabl on cathodes polarized in D_2O than on those polarized in H_2O (perhaps this is related to the greater equilibrium gas pressure for equivalent composition in the Pd-D system), and was different in appearance on materials from different sources These observations may be explained by the old idea[17] tha microfissures, or rifts, develop in palladium to release the mechanical strains resulting from the heavy loading of hydrogen together with the assumption that any such effect would dep on the stress state of the metal surface and its microstructure

Lithium was present in the surface layer on cathodes used in LiOD, and analysis by secondary-ion mass spectrometry (SIMS) apparently showed a concentration profile extending about 1 μm into the metal SIMS images showed that the lithium was not uniformly distributed, however, and it seemed likely that it was trapped in microfissures in the surface layer

Surface analysis showed a number of other species on and in the surface layer, notably small quantities of platinum and traces of copper, zinc, iron, lead and silicon platinum would have originated from the anode and silicon from the glass container No doubt the majority of the other contamination would have come from the solution the levels found (a few atom per cent, confined to the surface layers) were consistent with deposition by extended electrolysis from a solution o concentration around 10^{-9}-10^{-10}M One possible criticism is that low levels of such deposition could poison any essentia catalytic activity we therefore used pre-electrolysis in several experiments, in an attempt to lower the surface contamination We either made repeat experiments on the same solution, simply changing the cathode, or treated the solution beforehand in a separate cell

Because of claims that an unusual mechanism might lead t

TABLE 2 Measured neutron and γ ray emission rates for cold fusion electrolytic cells

a) Neutron emission rates

	Cathode				Details of electrolysis			
Material	Mass (g)	Surface area (cm^2)	Electro yte	Anode	Current (mA)	Typical voltage	Duration (h)	Measured neutron yield[w] ($n\ sec^{-1}$)
Pd rod 2 mm[a]	3 4	5 7	0 1MLiOD	Pt wire	360	5	914	These cells monitored initially by low efficiency n detectors with lower detection limit of ~100 s^{-1} Later moved to a dual cavity neutron detector[10] with lower detection limit ~2 s^{-1} No detected n emission
Pd wire 1 mm[a]	0 94	3 1	0 1M LiOD	Pt wire	200[r]	4	916	
Pd wire 1 mm[b]	0 94	3 1	0 1M L OD	Pt wire	200	4	916	
Pd wire 1 mm[b]	1 4	4 7	0 1M LiOD	Pt wire	300[s]	5	917	
Pd wire 1 mm[c]	0 47	1 6	0 1M LiOD	Pt wire	750	11	856	
Pd plate 1 mm[d]	7 5	13 5	1M LiOD	Pt sheet	2 000	10	307	
Pd foil	0 5	8 0	0 1M LiOD	Vitreous C	400-120[t]	4-20	142	
Pd/22Ag[e]	1 6	3 8	0 1M LiOD	Pt wire	380	5	859	
Pd/22Ag[e]	1 6	3 8	0 1M LiOD	Pt wire	380[u]	5	547	
Pd foil[f]	0 075	1 0	0 1M LiOD	Pt wire	30	3	87	−0 019 ± 0 042
Pd foil[g]	0 075	1 0	0 1M LiOD	Pt wire	250	7	6	0 00 ± 0 08
Pd foil[h]	0 075	1 0	0 1M LiOD +17 mM Na_2S	Pt wire	250	7	16	0 04 ± 0 11
Pd foil[i]	0 075	1 0	0 1M LiOD	Pt wire	250	8	16	0 005 ± 0 063
Pd foil[j]	0 075	1 0	0 1M LiOD	Pt wire	250	8	16	0 051 ± 0 061
Pd foil[k]	0 075	1 0	0 1M LiOD	Pt wire	1 000	15	56	0 068 ± 0 061
Pd foil	0 63	8 0	0 1M LiOD	Pt foil	1 000	14	17	−0 110 ± 0 082
Pd foil	0 63	8 0	0 1M LiOD	Pd foil	1 000	18	22	0 012 ± 0 079
Pd ribbon[l]	0 45	0 92	0 1M LiOD	Pd foil	600[v]	15	110	−0 063 ± 0 096
Pd foil	0 60	8 0	0 1M LiOD	Au wire	1 000	12	43	0 068 ± 0 058
Pd pellet[m]	3 0	2	0 1M LiOD	Pt foil	650	18	68	0 007 ± 0 042
Pd pieces[n]	4 4		0 1M LiOD	Pd foil	500	8	88	0 012 ± 0 033
Pd pieces	4 4		0 1M LiOD	Pd foil	500	15	88	0 003 ± 0 046[x]
Ti foil	0 038	1 0	0 1M D_2SO_4	Au wire	250	5	34	−0 091 ± 0 054
Ti foil	0 038	1 0	0 1M D_2SO_4	Au foil	1 000	5	3	−0 22 ± 0 12[y]
(continued)			0 1M D_2SO_4 +0 02M $PdCl_2$		1 000	5	4	−0 22 ± 0 12[y]
Ti rod	2 2	4 3	Jones[q]	Au foil	100	4	3	−0 06 ± 0 15[y]
(continued)					500	6	2	−0 42 ± 0 21[y]
Ti granules[o]	0 5		0 1M D_2SO_4	Pt wire	250	7	22	0 00 ± 0 072
Ti granules	0 5		0 1M D_2SO_4 +3 mM $Na_4P_2O_7$	Pt wire	250	7	66	0 044 ± 0 054
Ti granules	40		0 1M D_2SO_4	Pt wire	250	25	22	−0 009 ± 0 058
Ti granules	40		0 1M D_2SO_4 +3 mM $Na_4P_2O_7$	Pt foil	250	25	22	−0 010 ± 0 060
Ti granules	17		Jones	Au foil	500	26	24	0 021 ± 0 054
Ti granules								
Ti/6Al/4V	2 8	15 0	0 1M D_2SO_4	Pt wire	670	5	87	−0 003 ± 0 031[x]
Ti/6Al/4V[p]	2 7	14 3	0 1M D_2SO_4	Pt wire	660	5	19	0 031 ± 0 044[x]
TiFe granules	1		0 1M D_2SO_4	Pt foil	250	8	24	0 12 ± 0 06
$CeAl_2$ granules	11		0 1M LiOD	Pt foil	500	22	22	−0 051 ± 0 056
UPt_3 granules	57		0 1M LiOD	Pt foil	500	20	94	0 10 ± 0 06

(b) γ ray emission rates

	Cathode				Details of electrolysis			
Material	Mass (g)	Surface area (cm^2)	Electrolyte[z]	Anode	Current (mA)	Typical voltage	Duration (h)	Measured 5 488 keV γ ray yield[aa] ($\gamma\ s^{-1}$)
Pd foil	0 58	7 5	0 1M LiOH	Pt foil	450	9	45	$(-4\ 9 \pm 7\ 43) \times 10^{-3}$
Pd foil	0 74	9 5	0 1M LiOH	Pt foil	510	9	164	−0 011 ± 0 004
Pd sheet	7 7	12 9	0 1M LiOH*	Pt foil	200	4 6	50	−0 017 ± 0 012
Pd rod	3 1	2 3	0 1M LiOH	Au foil	1 000	26	17	0 008 ± 0 011
Ti foil	0 089	2 0	Jones*	Au foil	320	5	42	−0 022 ± 0 011
Ti foil	2 2	32	Jones	Au foil	1 000	4	12	−0 015 ± 0 034
Ti foil	2 2	32	Jones	Au foil	1 000	4	6	0 012 ± 0 007
Ti granules	25		Jones	Au foil	710	13	8	0 032 ± 0 036
Ti granules	25		0 1M H_2SO_4	Au foil	400	20	25	−0 019 ± 0 018
Ti granules	25		0 1M H_2SO_4	Au foil	765	16	16	0 017 ± 0 022
UPt_3 granules	42		0 1M LiOH	Pt foil	300	31	166	$(3\ 1 \pm 6\ 0) \times 10^{-3}$

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[a] Cells provided by M Fleischmann Cathodes analysed for H D after use—results H/D 0 01 0 02 D/Pd 0 84 0 72

[b] Cathode wound into a tight spiral Examined initially in the large n detector without shuttle for 147 h with estimated detection limit 1 n s^{-1} and a further 5 h with detection limit 0 2 n s^{-1} Surface of cathode then rubbed with S before further use

[c] Cathode cleaned with emery paper after 300 h

[d] Cathode examined initially in the large n detector without shuttle in 0 1M LiOD for 183 h estimated detection limit 1 n s^{-1} Abraded with 400-grit SiC paper before use

[e] Tubes from D storage system for Tokamak

[f] Cathode analysed for H D after use—results H/Pd 0 03 D/Pd 0 80

[g] Electrolyte of the above run reused

[h] Cathode and electrolyte of the above run reused Na_2S added as concentrated aqueous solution

Foil vacuum degassed 1 000 °C before use Analysed for H D after use—results H/Pd 0 02 D/Pd 0 83

[j] Foil dipped in Na_2S (concentrated solution) before use Analysed for H D after use—results H/Pd. 0 05 D/Pd 0 80

[k] Cathode cut in half and analysed for H D after use—results H/Pd 0 02 0 02 D/Pd 0 85 0 78

[l] Melt spun ribbon provided by Johnson-Matthey Technology Centre

[m] Arc remelted twice electrolytically charged for 1 month in 0 1M LiOD then frozen in liquid nitrogen dipped in concentrated Na_2S solution and transferred to n counting cell

[n] Four 1-2-mm-thick discs of different types of Pd (Johnson-Matthey) spot welded to Pd wires plus strained Pd Wire

[o] Electrolyte reused after a previous run with a Ti cathode and Pt anode

[p] Material heated to 900 °C then quenched in water before use

[q] AuCN in Jones electrolyte replaced with $NaAuCl_4$

[r] Current on for 36 h then changed between 200 mA and 20 mA every hour

[s] Current on for 36 h then changed between 300 mA and 30 mA every 5 min

[t] Current changed slowly over range shown during electrolysis period as anode disintegrated

[u] Current on for 36 h then cycled off for 10 h and on for 2 h for period of 270 h Then 5 000 s at 380 mA cathodic and 4 000 s at 10 mA anodic for rest of period

[v] Current cycled on/off every 2 h Neutron yield is for on cycle

[w] The errors assigned (1σ calculated over full run duration) vary somewhat because in an attempt (mostly in the earlier stages of the programme) to cover as wide a range of cell configurations as possible pairs of unpowered + unpowered and powered + unpowered runs were not always carried out and consequently allowance has to made for slight differences in overall cosmic ray neutron detection efficiencies caused by slight differences between the two nominally identical cells

[x] These data were obtained using a different data acquisition system to drive the shuttle and accumulate data set up to look for neutron bursts[10]

[y] Cells exchanged every 5 min by hand

[z] All electrolytes used for γ ray work were 50 50 H_2O D_2O except * which were 41 59 H_2O D_2O

[aa] For the present measurements the system was calibrated with a set of standard γ ray sources and a $^{238}Pu/^{13}C$ (α nγ) source emitting 6 129 keV γ rays The cells were positioned such that their cathodes were as close as possible to the detector crystal and the detection efficiencies were calculated by integrating previously measured[21] point efficiency functions over the cathode volume Peaks were searched for at 5 488 4 977 and 4 466 keV corresponding to the expected location of the full energy single escape and double escape peaks using the method recommended in ref 22

nuclear reactions proceeding predominantly non-radiatively to ^4He, four cathodes (Table 2, first four entries) were analysed by vacuum fusion/mass spectrometry. The high vapour pressure of palladium at the melting point caused difficulties, so internal standards were prepared by ion implantation of 10^{13} and 10^{15} atoms of ^4He into samples cut from the cathodes. Detection limits for ^3He and ^4He determined in this way were $\sim 8 \times 10^{10}$ atoms per sample—$(1\text{–}10) \times 10^{11}$ atom g^{-1}. We found no ^3He or ^4He. The expected level, if fusion had been occurring at the rate reported in ref. 1, was $\sim 10^{16}$ atom g^{-1}.

Evolution of hydrogen at a titanium cathode resulted, as is well known[13], in a dense network of hydride precipitates, observable by standard metallographic methods, penetrating below the surface. In 0.1M D_2SO_4 electrolyte at 100 mA cm^{-2}, the network penetrated ~30 μm in 1 h. Precious-metal deposition inhibited the electrolytic uptake, presumably by lowering the overvoltage and promoting gas evolution. Electrolysis in the 'brew' used by Jones *et al.*[2] resulted in hydride precipitates confined to the grain boundaries; other experiments showed that the presence of $PdCl_2$ in the electrolyte caused this effect, presumably as a consequence of palladium plating on the cathode. It is clear that expressions of the fusion rate that assume that the cathode has composition TiD_2 are completely misleading: metallography shows that the number of deuterium pairs is far fewer, hence the claimed fusion rate per deuterium pair is far higher than the figure given, and so the results are even more difficult to reconcile with expectations than had been implied[2].

Discussion

The interest in cold fusion has generated a large number o neutron counting experiments. It is well known that, in genera it is inadvisable to measure the signal + background and bac ground of counting measurements at different times and i different physical locations (as in ref. 1), because of unexpecte systematic variations. This is especially true for low-count-rat experiments. Compensating for variation of the background rat and assessing appropriate errors for the procedure chosen ar particular problems. The work of Jones *et al.*[2] can be criticize for such errors[18]. It is notable that in ref. 2, only one run i fourteen showed a significant effect and then only because th particular run was assigned a smaller counting error than th others. Here we have attempted to minimize, by the shuttl procedure, uncertainty about background and counter variabil ity and about error calculation. Given some of the more spec tacular claims that have been made, we note that further cautio is advisable because of the notorious sensitivity of $^{10}BF_3$ an ^3He proportional counters to humidity and of counter-amplifie systems to earth loops. In our work the neutron detectors wer segmented, and the relationship between signals from the seg ments was well known, so spurious effects giving inconsisten signals from the segments could be identified.

Failure to reproduce the effects has been attributed by som to the need for rigorous exclusion of hydrogen and claims hav been made that palladium electrodes must be cast and carefull degassed before use, to remove all traces of carbon and hydroge impurity which might decorate dislocations or other high-energ

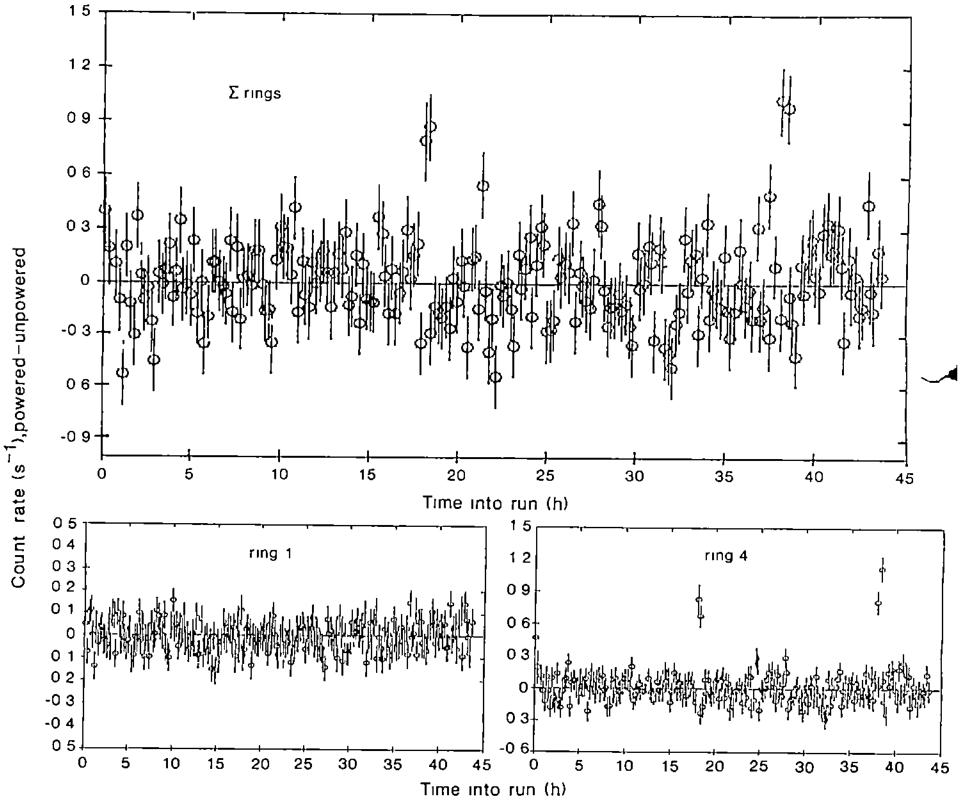

FIG. 3 Data from a typical run on the large high-efficiency neutron detector. These show the differences between count rates for powered and unpowered cells resulting from successive alternate shuttle positions for 40 g of titanium granules in 0.1M D_2SO_4. The errors shown are 1σ errors for each five-minute counting period. In addition to results from the detector as a whole, results from two of the five rings of 2-inch diameter 107-cm active length $^{10}BF_3$ proportional counters are also shown. The counts in rings 2, 3 and 5 (not shown) were very similar to those in ring 1. The apparent bursts were seen only in ring 4 and are therefore a spurious effect. A tube (90-mm inner diameter) passes through the centre of the detector, and the shielding consists of 6 inches of borated resin, 1 mm of cadmium and 2 inches of lead. The neutron detection efficiency is high and is largely independent of neutron energy, varying from 48% for 0.5-MeV (Am/Li) neutrons to 40% for 4.2-MeV (Am/Be) neutrons. The background count rate is 4-5 count s^{-1}, mostly neutrons from cosmic rays. Each of the five rings of counters has its own independent pre-amplifier, pulse-shaping amplifier and discriminator, and the mean energy of neutrons counted can be obtained from the ratio of counts in the outermost ring of counters (ring 5) to counts in the innermost (ring 1). Because neutron counts are distributed over five rings, and because of the 135-μs-mean time to capture, neutrons emitted simultaneously from a source as a burst are counted separately (as seen with the ^{252}Cf source, for example). The pre-amplifiers, high voltage components and insulators are all contained within a desiccated electrically screened box, and we eliminate earth loops and induced electromagnetic pick-up ('aerial') effects from external coaxial-cable runs to the PDP-11/45 data-acquisition computer by using isolating high-frequency pulse transformers and by winding the coaxial cables many times around ferrite rings. The neutron detector and its immediately associated electronics are located in a temperature-controlled air-conditioned blockhouse with two-foot-thick concrete walls and roof.

ites (ref 19 and R. A. Huggins, Workshop on Cold Fusion, anta Fe, May 1989): this is however inconsistent with the ostulate of a 'fusion' origin for the effect because, at low nergies, p-d fusion is expected to be significantly faster than -d fusion[3]. Furthermore, the original reports[1,2] did not mention ny special precautions to exclude atmospheric water vapour part from careful covering of the electrolytic cells. We followed imilar procedures, and found that degradation of the heavy ater by exchange with atmospheric moisture occurred quite apidly. Alternatively, it is claimed that it is essential to maintain high current density for a considerable period (A. J. Appleby, Vorkshop on Cold Fusion, Santa Fe, May 1989) although it is ot completely clear whether these latter claims are in fact eproductions of the effect reported in ref. 1 or are something ifferent. In our neutron counting experiments, fresh dislocaions were introduced by plastic deformation, some counting xperiments were carried out at current density as high as A cm^{-2} (Table 2) and in one experiment in the isothermal alorimeter a high current density was maintained for a considerble period (Table 1). Trace deposition of platinum on the athode, supposedly causing a lowering of the overpotential for euterium evolution and hence a lowering of the attainable euterium level in the cathode, has also been suggested as an xplanation for irreproducibility (M. Fleischmann, personal ommunication). We observed no effect when we used palladium nodes in our neutron counting experiments.

Timescales for hydrogen and deuterium loading consistent ith the expected diffusion time (x^2/D where the diffusion oefficient, $D = 10^{-7}$ cm^2 s^{-1} and x is the radius or half-thickness f the specimen) were measured[10] and nuclear counting and alorimetric experiments were always conducted over periods uch longer than this. Furthermore, in the process of electrolytic oading of palladium, there will clearly be a concentration radient, a moving phase boundary and the outer atomic layers f the metal will be saturated (possibly supersaturated) with euterium[17]. It might be expected, therefore, that sufficiently ensitive equipment would detect any fusion process well before e material is completely loaded. Because our neutron detection ensitivity was $\sim 10^5$–10^6 times greater than that of Fleischmann t al.[1], it seems unlikely that any greatly enhanced fusion process ssociated with the absorption of deuterium into palladium, iving rise to neutron emission, is occurring. We are, of course, ware that it is always possible to construct essentially untestable eories involving hypothetical special conditions of the metal r of its surface. Careful characterization of materials for which ositive results are claimed is therefore of great importance.

It has been argued that the neutron branch of the d-d reaction ight be completely suppressed in favour of the (t+p) branch nd it has been further argued (S. Pons, personal communicaon) that the tritium produced as a result of a nuclear process nside the electrode need not necessarily exchange with the lectrolyte and might not therefore be detected. However, the ther product of such a nuclear process, a high-energy proton, hould be detectable by its interaction with the lattice, includng neutron emission: we estimate, knowing the rate of energy ss of the protons and by comparison with (p, n) reaction ross-sections for neighbouring elements, a yield of $\sim 10^{-6}$ neutron per proton, implying a neutron yield in our ounting experiments of as much as 10^4 s^{-1} if fusion at the rate eported in ref. 1 were to proceed entirely through the (t+p) ranch.

In view of the rather large d-d separations in both PdD and iD_2, it might be argued that fusion requires some non-equilirium state in the lattice—perhaps at the α/β phase boundary n palladium or at the tips of the growing TiD_2 needles or at a attice defect—where the d-d or p-d separation could be greatly educed. Here we created non-equilibrium situations by pulsing he current but we detected no neutron emission (Fig. 4).

Some explanations of the apparent excess heat production ave emphasized recombination processes at catalytic metal surfaces[20]: apart from occasional explosions, however, which did not result in any detectable neutron emission, we found, by comparison of the volume of water added to maintain the cell volume with the electrolysis charge passed, that this was not a significant effect (in agreement with others[9]). Recombination in the gas space above the liquid, either on exposed cathode surface or catalysed by colloidal metal particles eroded from the cathode, might however account for some of the observations of bursts of heat recently reported (M. Fleischmann and S. Pons, Electrochemical Society Meeting, Los Angeles, May 1989). In discussing the claims in ref. 1, we prefer to focus on characteristics of the 'simple' Fleischman, Pons and Hawkins (FPH) calorimeters, because we have only observed small effects (at the level of the inherent uncertainties), which might mistakenly be claimed as arising from cold fusion, in the one type of calorimeter (FPH type) that has major calibration difficulties. Cells using the same electrode and electrolyte materials operated in calorimeters that did not have these problems exhibited none of these effects.

There are two points regarding the calibration that could have a profound effect on the apparent results obtained with FPH-type calorimeters: the first concerns when the calibration is performed and the second how it is performed. Concerning the first point, it seems from our work and that of Lewis *et al.*[8] that a calibration performed during the first 10,000 min of electrolysis could be seriously in error and lead to an erroneous conclusion that subsequently, rather than being in balance, the cells were exothermic. Concerning the second point, Fleischmann *et al.*[1] describe calibration using the internal-resistance heater, by measurement of Newton's-law-of-cooling losses. Typically, this procedure might involve the application of power to the heater while electrolysis was occurring, following the temperature-time trace until a steady state was obtained, then switching the heater off and following the cooling curve. This procedure gives an approximation to the differential calorimeter constant, $k_d = d(\Delta P)/d(\Delta T)$ (where P is power and T is temperature) at the operating temperature of the cell and can give rise to errors in two ways. First, because any calibration sequence would require

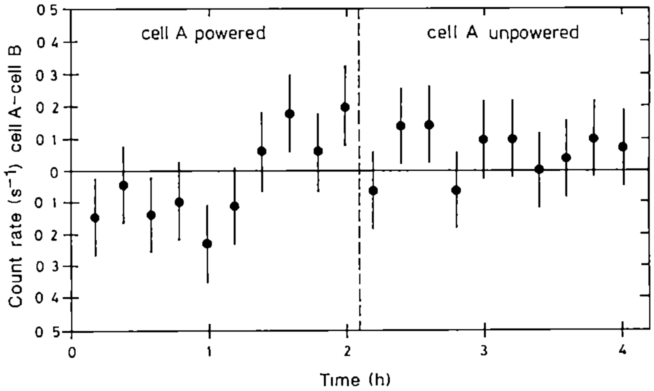

FIG. 4 Time dependence of neutron emission from an electrolytic cell with melt-spun Pd ribbon cathode and Pd foil anode, with power alternately turned on for deuterium loading and off for relaxation every 2 h by the data-acquisition computer: see Table 2 footnote *l*. The total length of the run was 110 h, and the sums of the 'on' and 'off' data are shown. After 90 h of running, we added 1 μM Pb^{2+} (to poison the surface by Pb deposition) to the solution. There was no difference between the data in the presence and absence of Pb^{2+}, and so data for the whole run was combined. This run was undertaken to enhance the appearance of the non-equilibrium effects discussed in the text. It is assumed that the ribbon would have had a high density of grain boundaries, dislocations and other lattice defects. Because the 'on' and 'off' periods were several times the characteristic diffusion time for the ribbon, it is assumed that the composition was cycling in the β-phase region between the fully loaded condition (D/Pd ≈ 0.83) and the limit of the (α + β) phase field (D/Pd ≈ 0.65).

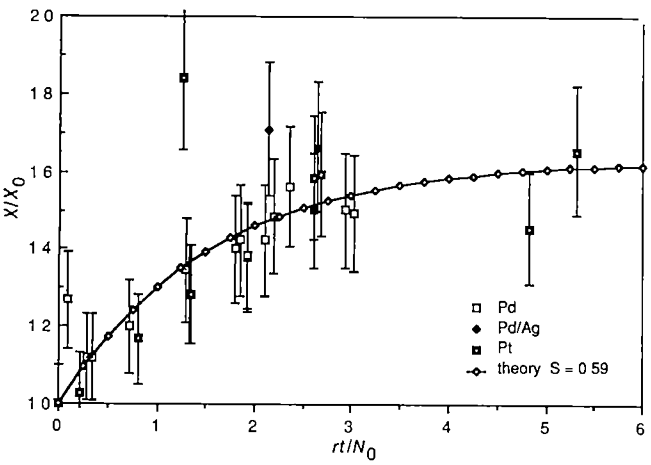

FIG. 5 Tritium enrichment by electrolysis in open cells at constant volum with both Pd and Pt cathodes: relative count rate X/X_0 against amount c electrolysis rt/N_0 (symbols defined in the text). Initial count rate (efficienc corrected) was 13.0±0.6 kBq ml^{-1}. Errors are 1σ. The Pd cells were th FPH calorimeters, sampled after 490-660 h, the cells in the first section c Table 2 (footnotes *a–e*) sampled at the end of the run and one cell fror the high-efficiency neutron detector—footnote *k* in Table 2. The line is th fit to equation (1).

5–10 h, extrapolation would be required to obtain the correct value at the reference liquid level: an estimated error of 20% or more could result. Furthermore, because the evaporation of the cell contents increases markedly with increasing temperature, the baseline slope would increase with increasing temperature and the effects of this sort of error would become correspondingly more marked: the claimed effects were indeed greatest in the cells run at the highest input power. Second, these calorimeters are nonlinear (equation (3), Fig. 2 legend), with the effect being significant when the temperature gradient is large: they cannot be described by a simple Newton's-law-of-cooling constant. If a differential calorimeter constant is used to derive the input power, the calculated output would be (from Fig. 2 legend, equation (3))

$$P_{\mathrm{app}} = k_{\mathrm{d}}\Delta T = (k_{\mathrm{sb},0} + k_{\mathrm{c}})\Delta T + 3(k_{\mathrm{sb},0}/T_0)(\Delta T)^2$$

so that, in comparison with the correct output (equation (3))

$$P_{\mathrm{app}} - P = 3k_{\mathrm{sb},0}(\Delta T)^2/2T_0)$$

If the power applied to the heater is significant compared with the electrolysis power then this error will be even greater. Back calculation from the data given in ref. 1 shows that the claime effects were largest for Joule input powers on the order of 6 W For our FPH-type calorimeters, this would have given $\Delta T \approx$ 50–60 °C. Therefore, had these cells been calibrated in this way an apparent heat excess on the order of 0.8–1 W would hav been observed. Our method of calibration (see Fig. 1 caption considerably reduced the effect of these sources of error. Th original report[1] was clearly preliminary in nature, and it i evident from the above that the claims made therein cannot b assessed in the absence of a detailed description of the experimental procedure used and of the methods used to compensat for the systematic errors inherent in the use of a simpl calorimeter.

We feel that our work has served to establish clear bound for the non-observance of cold fusion in electrolysis cells, unde carefully controlled and well understood experimental conditions and using well characterized materials. Further detail are given in ref. 10. Claims of observations of cold fusio ought now to meet similar standards of data analysis an materials characterization so that a proper assessment can b made. ☐

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1. Fleischmann, M., Pons, S. & Hawkins, M. *J. electroanal. Chem.* **261**, 301–308 (1989); erratum **263**, 187 (1989).
2. Jones, S. E. *et al. Nature* **338**, 737–740 (1989).
3. Koonin, S. E. & Nauenberg, M. *Nature* **339**, 690–691 (1989).
4. Sun, Z. & Tomanek, D. *Phys. Rev. Lett.* **63**, 59–61 (1989).
5. Leggett, A. J. & Baym, G. *Nature* **340**, 45–46 (1989).
6. Gai, M. *et al. Nature* **340**, 29–34 (1989).
7. Zeigler, J. F. *et al. Phys. Rev. Lett.* **62**, 2929–2932 (1989).
8. Lewis, N. *et al. Nature* **340**, 525–530 (1989).
9. Cunnane, V. J., Scannell, R. A. & Schriffrin, D. J. *J. electroanal. Chem.* **269**, 163–174 (1989).
10. Williams, D. E. *et al. Harwell Report AERE R* 13606 (HMSO, London, 1989).
11. Edwards, G., Findlay, D. J. S. & Lees, E. W. *Ann. Nucl. Energy* **9**, 127–135 (1989).
12. Lees, E. W., Patrick, B. H. & Bowey, E. M. *Nucl. Instrum. Meth.* **171**, 29–41 (1980).
13. Fouroulis, Z. A. *J. electrochem. Soc.* **128**, 219–221 (1981).
14. Ostlund, H. G. & Werner, E. in *Tritium in the Physical and Biological Sciences* Vol. 1, 95–104 (Int. Atomic Energy Agency, Vienna, 1962).
15. Kaufmann, S. & Libby, W. F. *Phys. Rev.* **93**, 1337–1334 (1954).
16. von Buttlar, H., Vielstich, W. & Barth, H. *Ber. Bunsenges. phys. Chem.* **67**, 650–657 (1963).
17. Smith, D. P. *Hydrogen in Metals* (University of Chicago Press, 1948).
18. Carpenter, J. M. *Nature* **338**, 711 (1989).
19. Gittus, J. & Bockris, J. *Nature* **339**, 105 (1989).
20. Kreysa, G., Marx, G. & Plieth, W. *J. electroanal. Chem.* **266**, 437–441 (1989).
21. Croft, S. *Nucl. Instrum. Meth. Phys. Res.* **A281**, 103 (1989).
22. Anicin, I. V. & Yap, C. T. *Nucl. Instrum. Meth. Phys. Res.* **A259**, 525–528 (1987).
23. Savitzky, A. & Golay, M. J. E. *Anal. Chem.* **36**, 1627–1639 (1964).

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Induction of self-tolerance in mature peripheral B lymphocytes

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n transgenic mice, mature peripheral B lymphocytes in lymphoid follicles, like immature B cells, are rendered tolerant by encounter with self-antigen, provided receptor occupancy by self-antigen exceeds a critical threshold The tolerant state of the B cell is closely correlated with down-egulation of membrane IgM but not IgD antigen-eceptors Identical changes in antigen-receptor xpression occur in a subset of follicular B cells n nontransgenic mice, suggesting that clonally ilenced self-reactive cells are common in the eripheral B-cell repertoire

o maintain a state of immunological self-tolerance, potentially elf-reactive lymphocytes must be prevented from mounting nmune responses to autologous antigens In the case of T ymphocytes, irreversible clonal deletion of self-reactive cells as recently been shown to play an important role in maintaining elf-tolerance within the T-cell repertoire[1-5] Selective deletion f self-reactive T cells occurs within the thymus by a mechanism milar to that orginally proposed by Lederberg[6], whereby the nteraction between self-antigen and the T-cell receptor at an nmature stage in T-cell differentiation aborts any further evelopment of the cell[7] Such a censoring mechanism is ffective in maintaining self-tolerance within the peripheral T-ell repertoire because the sequence and specificity of T-cell eceptors remains constant after receptor gene rearrangement[8-10] Self-tolerance in B-lymphocytes, however, poses an dditional problem because the antigen-binding sites of nmunoglobulins are further diversified after B cells have left bone marrow, by hypermutation of immunoglobulin genes in mature, peripheral B cells[11,12] Hypermutation of nmunoglobulin genes not only provides the extra diversity eeded for production of high-affinity antibodies to most foreign ntigens[11,12], but also results in generation of self-reactive B-cell ariants at a detectable frequency[13,14] To prevent production f high-affinity pathogenic autoantibodies by this 'second wave' f self-reactive B cells, a mechanism of self-tolerance is required hat can act at multiple stages of B-cell development

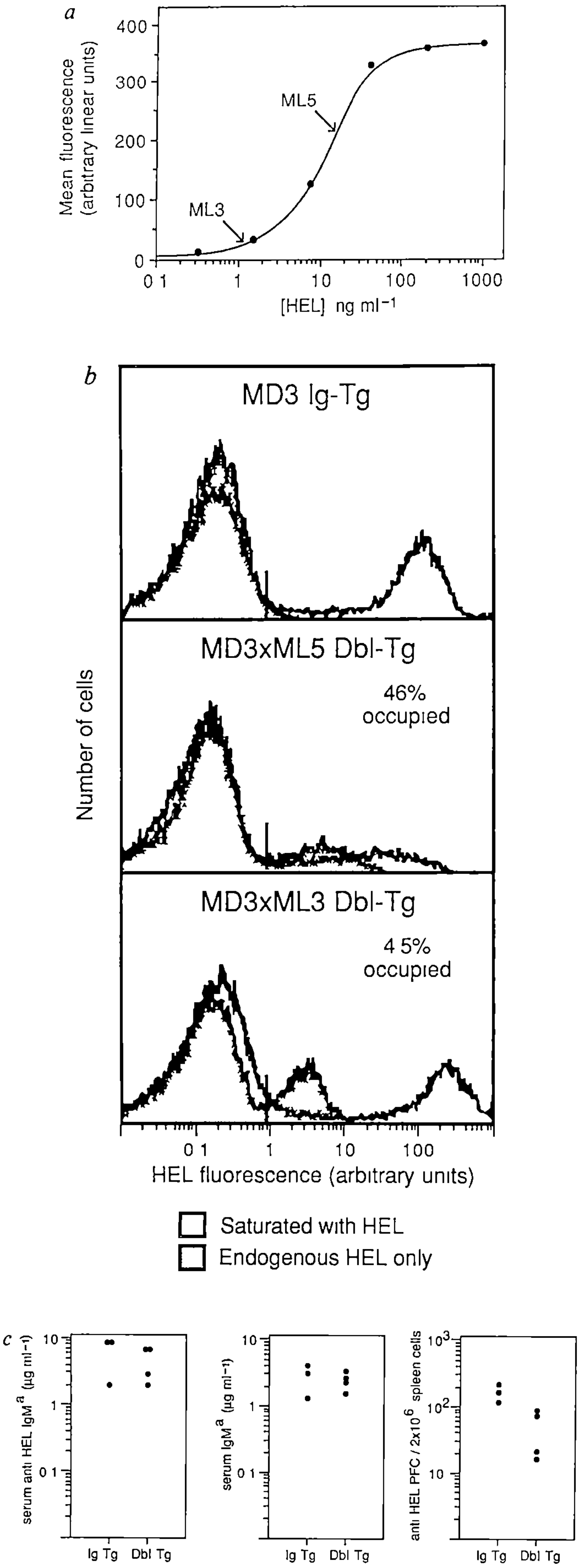
High-affinity antibody responses to xenogeneic or allogeneic roteins or cells have long been known to be restricted to pitopes that differ between immunogen and self, despite the resence of many cross-reactive epitopes on such antigens[15,16], nd this principle underlies the widespread practical use of pecies- or allele-specific antisera and monoclonal antibodies s T-cell 'help' is potentially available to any B cell capable of inding and presenting the immunogen[17], the restriction of the ntibody response to those epitopes on xeno- or alloantigens hat are not cross-reactive with self provides a clear indication hat self-reactive B cells are censored at some stage during the eneration of a high-affinity antibody response[16] Indeed, vidence for induction of tolerance at multiple stages of B-cell evelopment, including mature and memory B cells, has been epeatedly found in *in vivo* models of tolerance induction to exogenous antigens administered in an appropriate form[18-26] It has been difficult, however, to determine the fate of the unresponsive mature peripheral B cells in these models because of the low-frequency of antigen-specific B cells Also, the possibility remains that some examples of *in vivo* B-cell unresponsiveness may involve immunoregulatory mechanisms not directly related to self-tolerance, such as exhaustive differentiation into plasma cells, feedback effects due to antigen-antibody complexes and receptor blockade[20,21,26]

To increase the frequency of potentially self-reactive B cells, thereby allowing their fate to be traced *in vivo*, we have recently developed a transgenic mouse model in which hen-egg lysozyme transgenic mice were mated with anti-lysozyme immunoglobulin-transgenic mice to create 'double-transgenic' offspring[27] The anti-lysozyme B cells from these mice encountered lysozyme as a self antigen throughout their development within the bone marrow and in the periphery Despite this, the B cells were not deleted, but persisted in a functionally silenced state characterized by expression of high levels of membrane IgD but a 10-20 fold reduction in membrane IgM[27] The current paper describes the use of the same transgenic model to follow the fate of self-reactive B cells when they encounter self-antigen only at the mature stage of development, in peripheral lymphoid organs As observed previously in conventional *in vivo* models, mature follicular B cells in the transgenic mice were fully susceptible to induction of self-tolerance Also, the silenced state appeared identical to tolerance induced by encounter with self-antigen early in B-cell development, in that it required comparable levels of receptor occupancy, and was intimately linked to downregulation of membrane IgM but not IgD A significant proportion of IgD^+ follicular B cells in nontransgenic mice show an identical change in membrane IgM, indicating that clonally silenced self-reactive B cells may be frequent in peripheral lymphoid organs

Low-lysozyme double-transgenic mice

In previous experiments[27], functional silencing of anti-lysozyme B cells took place in double-transgenic mice derived from matings of two parental lines The first line, ML5, was the highest expressing of a series of transgenic lines bearing the hen-egg lysozyme (HEL) gene linked to the mouse metallothionein promoter The other parental line, MD3, was one of six lines bearing rearranged immunoglobulin heavy and light chain genes encoding high affinity anti-lysozyme IgM and IgD, the two isotypes being expressed by differential RNA-splicing from the V-μ-δ heavy chain transgene Lysozyme-binding B cells persisted in peripheral lymphoid organs of the resulting double-transgenic progeny, but they no longer secreted anti-lysozyme antibody and exhibited an altered phenotype, characterized by a 10-20 fold reduction in membrane IgM with no change in membrane IgD Developing B cells in the bone marrow of the double-transgenic mice encountered lysozyme shortly after expressing surface IgM, as bound lysozyme could be demonstrated on these cells (see below) and because down-regulation of membrane IgM was observed even on newly differentiated $B220^{low}$ bone marrow B cells (D Y Mason *et al*, manuscript in preparation)

In terms of B-cell receptor occupancy in the double-transgenic mice, the mean concentration of lysozyme in the serum of the



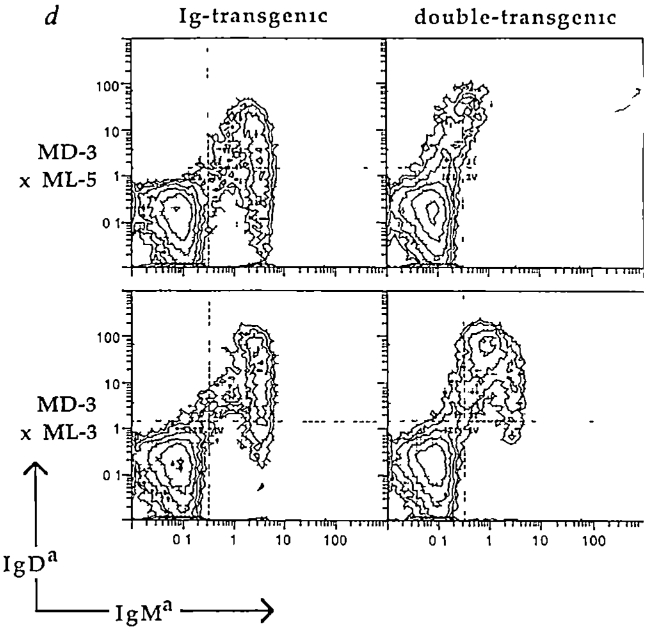
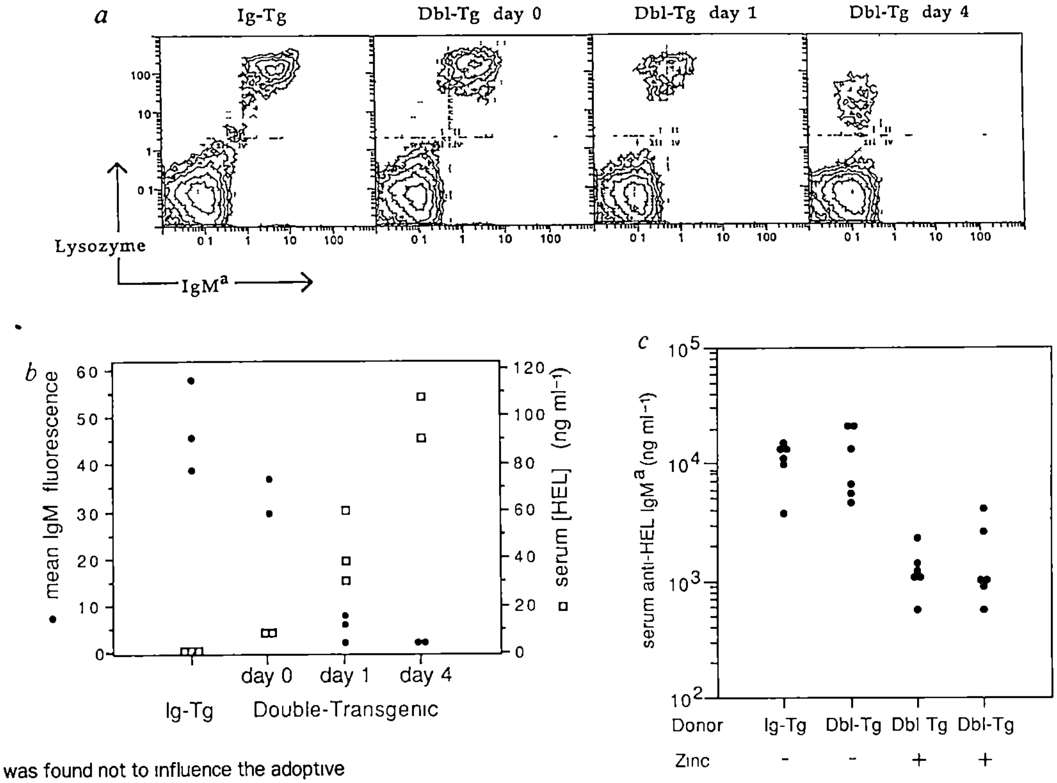
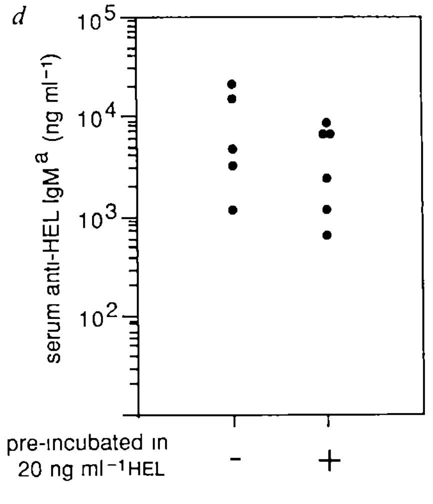


FIG 1 Lysozyme-binding B cells in ML3 × MD3 double-transgenic mice exhibit decreased antigen receptor occupancy (*a*, *b*), failure of B-cell silencing (*c*), and absence of IgM-downregulation (*d*) *a*, Binding of lysozyme to MD3 immunoglobulin-transgenic spleen cells *in vitro* titrated over a range of lysozyme concentrations, by measuring subsequent binding of a biotinylated second anti-lysozyme monoclonal antibody on the FACS *b*, Both immunoglobulin- and double-transgenic spleen cells exposed to saturating concentrations of lysozyme (unshaded histograms) or no lysozyme (shaded histograms) *in vitro* before second-step staining with the biotinylated anti-lysozyme monoclonal antibody Positively staining cells in the shaded histograms therefore reflect cells which have bound lysozyme *in vivo* before preparation and staining *in vitro* *c*, Spontaneous secretion of transgene-encoded (a-allotype) anti-lysozyme IgM measured in the serum of MD3 × ML3 double-transgenic mice and immunoglobulin-transgenic littermates by lysozyme-binding enzyme-linked immunosorbent assay (ELISA) (*c*, left panel) or by IgM^a allotype ELISA (*c*, centre), and by quantitating the number of anti-lysozyme IgM plaque-forming cells (PFC) in the spleen (*c*, right) Each point represents the value from an individual mouse *d*, Expression of transgene-encoded membrane IgM and IgD on spleen cells from MD3 × ML5 or MD3 × ML3 double-transgenic mice and immunoglobulin-transgenic littermates determined by two-colour FACS analysis Dots and contours are set respectively at 1 or more cells and 5, 10, 20, 40, 80, and 160 cells per 4 × 4 channel grid

METHODS Transgenic mice were derived from C57BL/6 founders as described[27] and maintained by backcrossing to C57BL/6 Lysozyme-binding was measured by incubating spleen or bone marrow cells with either no HEL or the indicated concentrations of HEL on ice for 25 min, followed by staining with biotinylated HyHEL9 and streptavidin-phycoerythrin as described previously[27] Logarithmic amplifiers on the FACS were calibrated using a series of standards, and the resulting nomogram used to convert mean fluorescence channel number to mean linear fluorescence Per cent receptor occupancy was then derived by determining the mean fluorescence of the positively staining cells, using the gates shown, in the absence of any added HEL (shaded histograms), and dividing this value by the mean fluorescence of the same population of cells when all available receptors were saturated with 200 ng ml^{-1} HEL (unshaded histograms) Anti-lysozyme IgM^a was measured at several dilutions from sera by ELISA on HEL-coated 96 well plates as previously, except that the assay was developed with biotinylated anti-IgM^a monoclonal antibody RS3 1 (ref 51) followed by avidin-alkaline phosphatase (Sigma) Absolute concentrations were determined relative to a standard curve of culture supernatant from SP2/0 cells transfected with the anti-HEL immunoglobulin genes Measurement of serum IgM^a allotype, anti-HEL direct plaque-forming cells, and two-colour FACS analysis were all as described previously[27] arental ML5 mice (17 4 ng ml^{-1}), and in ML5×MD3 double-ansgenic progeny, was close to that required to half-saturate ie anti-lysozyme immunoglobulin receptors expressed by MD3 mmunoglobulin-transgenic B-cells *in vitro* (Fig 1*a*), and *in vivo* Fig 1*b*) Because the lysozyme concentration in ML5×MD3 ouble-transgenic mice lies in the steepest portion of the receptor tration curve (Fig 1*a*), a relatively small reduction in lysozyme xpression would be expected to result in a large decrease in eceptor occupancy *in vivo* To examine the effect of such a ecrease in receptor occupancy on B-cell silencing, the same ne of immunoglobulin-transgenic mice, MD3, was mated with different line of metallothionein-lysozyme transgenic mice, IL3, which expressed an order of magnitude less lysozyme mean serum HEL, 1 4 ng ml^{-1})

In ML3×MD3 double-transgenic mice, only 4 5% of anti-ysozyme membrane immunoglobulin on splenic B-cells was ccupied with lysozyme (Fig 1*b*), compared with 46% ccupancy in ML5×MD3 double-transgenics A similar ifference in receptor occupancy was found on bone marrow B ells (data not shown), indicating that in both types of double-transgenic mice, lysozyme was encountered early in B-cell differentiation Comparison of these low-lysozyme double-transgenics with immunoglobulin-transgenic littermates revealed that, in contrast to the profound silencing of anti-lysozyme B cells observed in ML5×MD3 double-transgenic mice[27], there was little or no reduction in the concentration of transgene-encoded anti-lysozyme IgM in the serum of ML3×MD3 double-transgenics, and the number of cells secreting anti-lysozyme IgM in the spleen was only slightly lower than that of immunoglobulin transgenic littermates (Fig 1*c*) In addition to the failure of tolerance in ML3×MD3 double-transgenic mice, FACS analysis of their spleen cells revealed minimal down-regulation of membrane IgM on the anti-lysozyme B cells (less than twofold), compared with the 10-to 20-fold reduction in surface IgM on silenced B cells from ML5×MD3 double transgenic mice (Fig 1*d*) Conversely, the T-helper cell compartment in ML3 transgenic mice was tolerant to lysozyme (S Adelstein, unpublished results, see below), which is consistent with previous data showing a lower antigen concentration threshold for induction of unresponsiveness in T cells[18]





G 2 Zinc-induction of serum sozyme in adult MD3×ML3 ouble-transgenic mice is accom-anied by rapid downregulation of embrane IgM and induction of lerance MD3×ML3 double-ansgenic mice or immuno-obulin-transgenic littermates ere given drinking water con-ining 25 mM zinc sulphate for r 0, 1 or 4 days All mice were lled at the same time *a*, ysozyme-binding versus IgM^a rofiles of spleen cells obtained y two-colour FACS analysis *b*, lean IgM levels on lysozyme-inding B cells expressed in rbitrary linear units compared to erum lysozyme concentration in dividual animals at each time oint *c*, Antibody responses of plenic B cells from individual nmunoglobulin-transgenic, unin-uced double-transgenic or 4-day duced double-transgenic mice leasured in adoptive transfer, e dots represent the concentra-of serum anti-HEL IgM^a in dividual recipients of spleen ells from two different zinc-nduced double-transgenic mice n other experiments, zinc exposure was found not to influence the adoptive esponse of immunoglobulin-transgenic spleen cells (data not shown) *d*, eceptors on MD3 immunoglobulin-transgenic spleen cells were artificially locked' by exposure to a half-saturating concentration of lysozyme 20 ng ml^{-1}) on ice for 1 h before adoptive transfer

IETHODS All transgenic mice were maintained on a C57BL/6 background, nd used at 8–14 weeks of age Serum HEL was measured by capture LISA[52] using 96-well plates coated with the monoclonal antibody HyHEL10 erum was tested at a dilution of 1 5 or 1 10, and bound HEL revealed with iotinylated monoclonal HyHEL9 followed by avidin-alkaline phosphatase bsolute values were derived from a standard curve of purified HEL FACS nalysis was performed as previously described, staining for HEL-binding ncluded a first step exposure to 200ng ml^{-1} HEL to saturate any remaining ree receptors Mean IgM^a on HEL-binding cells was measured by staining vith fluoresceinated RS3 1 antibody and conversion to mean linear fluorescence using a calibrated logarithmic amplifier as described in Fig 1 Adoptive ransfer was carried out as previously described[27], transgenic spleen cells 10^4) were injected intravenously together with 5×10^6 HRBC-primed 57BL/6 cells and 2×10^8 HEL-HRBC into 750 rad irradiated C57BL/6 ecipients Serum anti-HEL in the recipients was measured 7 days later as lescribed in Fig 1

Induction of lysozyme in adult mice

The findings in the low-lysozyme double-transgenic mice suggested that tolerance induction and the accompanying down-regulation in surface IgM depends on exposure of anti-lysozyme B cells to a threshold concentration of lysozyme which corresponds to a receptor occupancy of between 4 5% and 45% of surface immunoglobulin They do not indicate, however, whether such a tolerance threshold is unique to immature B cells[26], nor whether mature B cells would in fact be triggered rather than silenced if above-threshold concentrations of lysozyme were encountered only after nontolerant B cells had matured in adult animals To answer these questions, the serum lysozyme concentration in ML3 × MD3 double-transgenic mice was elevated above the putative threshold by exploiting the heavy metal inducibility of the metallothionein promoter[28] Adult ML3 × MD3 low-lysozyme double-transgenic mice were given drinking water containing 25 mM zinc for 0, 1 or 4 days As a result, the serum lysozyme concentration was elevated to 29-60 ng ml^{-1} in mice exposed to zinc for 1 day, and reached 89-108 ng ml^{-1} following exposure for 4 days (Fig 2*b*) In parallel with the rise in lysozyme concentration, membrane IgM on HEL-binding spleen cells from these mice was partially downregulated by day 1 (Figs 2*a*,*b*), and by day 4 of zinc exposure had fallen to a level comparable with that observed in the ML5 × MD3 double-transgenic mice Importantly, at the intermediate timepoint there was no evidence for a bimodal distribution of membrane IgM levels expressed by the B cells (Fig 2*a*) and at no stage was there a reduction in the number of B cells, excluding the possibility, therefore, that pre-existing $\mathrm{IgM}^{\mathrm{hi}}$ cells were simply being replaced by a discrete population of $\mathrm{IgM}^{\mathrm{low}}$ cells

The functional status of anti-lysozyme B cells from the zinc-induced double-transgenic mice was assessed by adoptive transfer (Fig 2*c*) In contrast to B cells from immunoglobulic-transgenic or uninduced ML3 × MD3 double-transgenic mice, B cells from the mice exposed to zinc for 4 days responded poorly when challenged with lysozyme coupled to a foreign carrier (horse red blood cells, HRBC) following adoptive transfer into irradiated nontransgenic recipients together with HRBC-primed helper cells In other words, the zinc-induced rise in serum lysozyme concentration and B-cell receptor occupancy led to changes in receptor phenotype and function in mature, peripheral B lymphocytes from adult ML3 × MD3 animals which were identical to those described previously in silenced B cells from ML5 × MD3 double-transgenic mice The functionally silenced state of the B-cells cannot be accounted for simply by 'blocking' of antigen receptors with lysozyme for two reasons first, less than 50% of the receptors were occupied in ML5 × MD3 double-transgenic mice (Fig 1*b*), and second, artificially 'blocking' half the receptors with lysozyme *in vitro* before adoptive transfer failed to alter the responsiveness of immunoglobulin-transgenic cells (Fig 2*d*)

Transfer of nontolerant B cells

The apparent susceptibility of mature peripheral B cells to IgM receptor down-regulation and silencing was examined in a second way, by transferring nontolerant spleen cells from immunoglobulin-transgenic mice into irradiated ML5 (high lysozyme) transgenic recipients or nontransgenic littermates (Fig 3) The possibility of a donor T-cell response to recipient lysozyme was minimized by using C57BL/6 strain donors and recipients, which are low responders to lysozyme[29] Between 5 and 10% of the injected lysozyme-binding B cells homed to the spleen of both types of recipients In the case of lysozyme transgenic recipients, the cells exhibited a 10-fold down-regulation of surface IgM as early as 20 h after transfer (Fig 3*a*) and persisted in this state for at least 10 days (data not shown) The functional capacity of the transferred B cells was examined by co-injecting the immunoglobulin-transgenic spleen cells with spleen cells from nontransgenic donors primed to HRBC to act

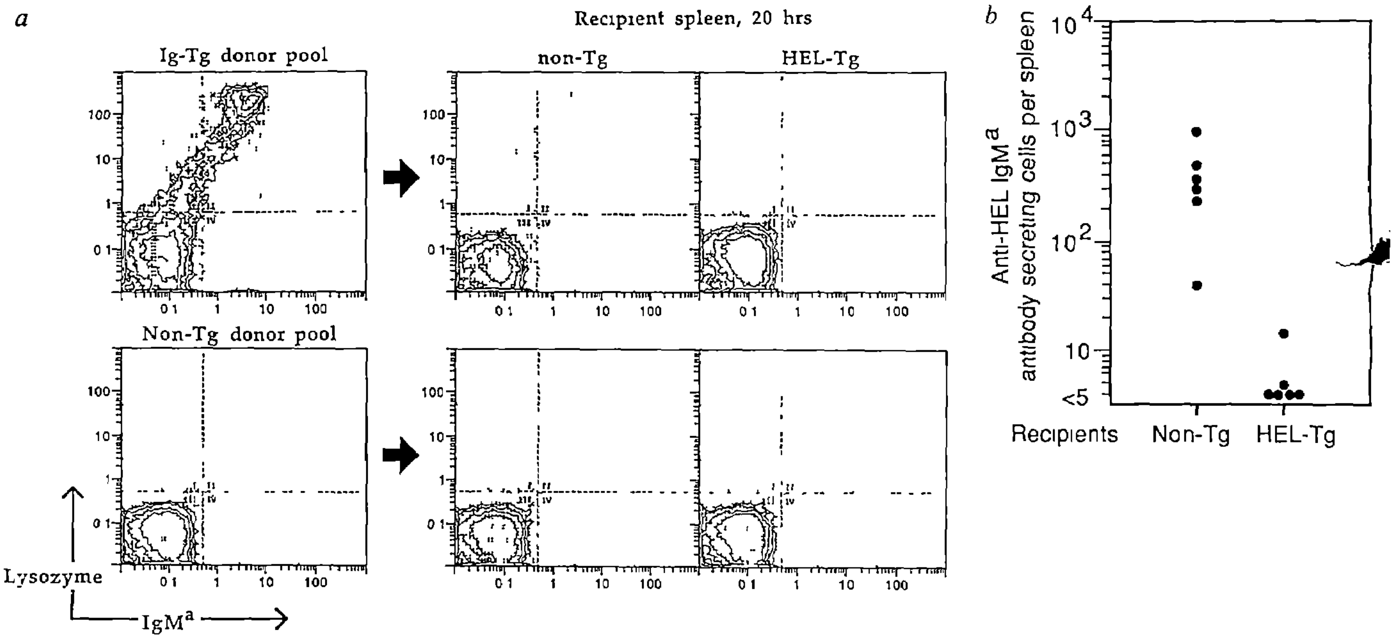


FIG 3 Transfer of immunoglobulin-transgenic spleen cells into ML5 HEL-transgenic recipients leads to rapid downregulation of membrane IgM and induction of tolerance *a*, For FACS analysis, 10^7 spleen cells from adult MD3 immunoglobulin-transgenic mice or nontransgenic controls were injected into 750-rad-irradiated ML5 transgenic mice or nontransgenic littermates, and recipient spleen cells analysed by two-colour FACS for HEL-binding against IgM at 20 h (*a*), 2 5 days or 10 days after transfer Duplicate recipients at each timepoint gave similar results to *a* *b*, For antibody responses, 10^6 immunoglobulin-transgenic cells were co-injected with 5×10^6 HRBC-primed C57BL/6 spleen cells into irradiated ML5-transgenic mice or nontransgenic littermates, left for two days, and then challenged with 2×10^8 HEL-HRBC intravenously Antibody responses in individual recipients were measured as anti-HEL $\mathrm{IgM}^{\mathrm{a}}$ antibody-secreting cells in the spleen by spot-ELISA[50] five days after challenge

METHODS All donors and recipients were adult animals of C57BL/6 genetic background Antibody secreting cells were measured by spot ELISA[53], in 24-well plastic trays coated with 1 mg ml^{-1} HEL Spots of bound antibody were revealed with biotinylated anti-$\mathrm{IgM}^{\mathrm{a}}$ antibody RS3 1 followed by avidin-alkaline phosphatase a source of helper T cells Two days after cell transfer, the cipient mice were challenged with lysozyme coupled to HRBC HEL-HRBC) In contrast to the good response obtained after parking' in nontransgenic hosts, immunoglobulin-transgenic B lls parked in HEL-transgenic recipients failed to mount an ntibody response, as measured either by antibody-secreting ells in the spleen (Fig 3*b*) or by serum antibody titres (data ot shown)

olerance and IgM-down-regulation

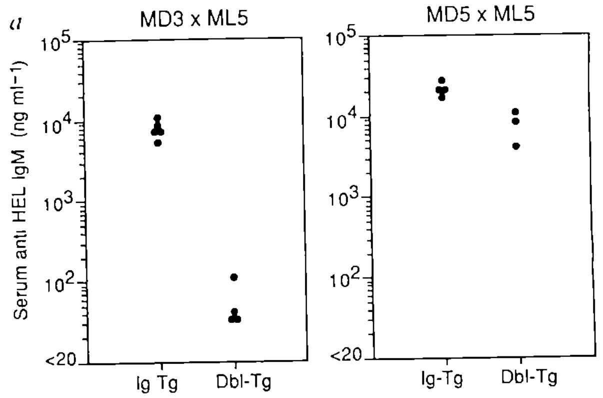
he results from the low-lysozyme double-transgenic mice, the ffects of zinc induction and the cell transfer experiments emonstrate a good correlation between selective down-regulaon of membrane IgM but not IgD and clonal silencing of the cell The lack of down-regulation of membrane IgD cannot e explained in terms of failure of IgD to bind lysozyme as B ells from lines of immunoglobulin-transgenic mice expressing nti-lysozyme IgD alone bind lysozyme as efficiently as B cells rom immunoglobulin-transgenic mice expressing IgM-alone, nd B cells from double-transgenic mice expressing IgM alone how a more marked decrease in lysozyme binding which is directly proportional to the down-regulation of membrane IgM unpublished observations) Thus the 4–10 fold decrease in ysozyme binding that accompanies the 10–50 fold down-regulaion of IgM in tolerant double-transgenic mice expressing IgM nd IgD (Figs 2*a*, 4*b*) can best be explained by binding of ysozyme to unchanged levels of membrane IgD The degree of eduction in lysozyme binding observed may reflect both the ct that a subpopulation of the B cells do not express IgD (see low), and that in the absence of any down-regulation, IgM may be expressed at higher density than IgD

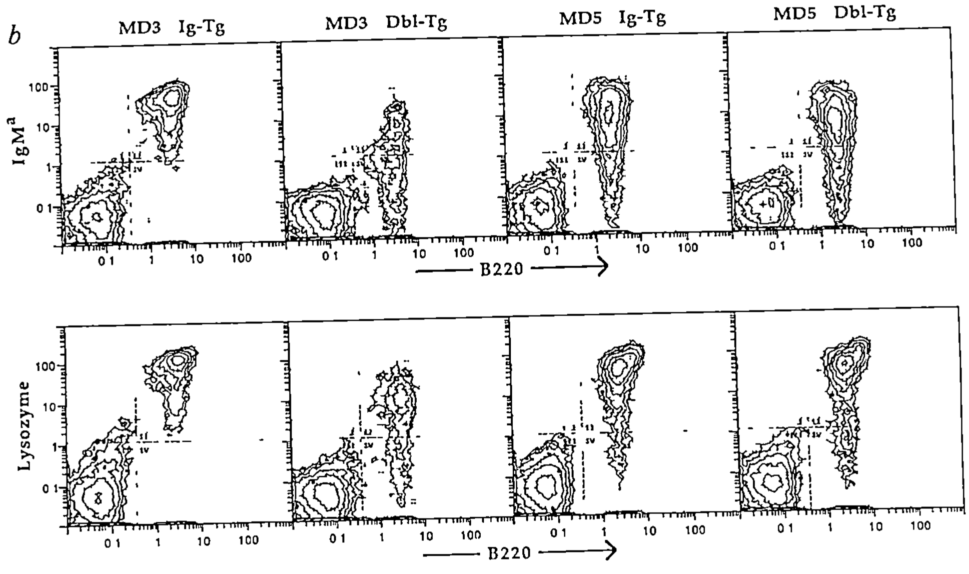
A close relationship between selective down-regulation of membrane IgM and B-cell tolerance was also found in a eciprocal experimental approach, which involved mating different lines of anti-lysozyme IgM+IgD immunoglobulin-ransgenic mice to the original (high lysozyme) ML5 transgenic ine Four out of the five double-transgenic combinations yielded imilar results to ML5×MD3 (a detailed comparison of all the ines will be described elsewhere) Double-transgenic offspring from the fifth immunoglobulin-transgenic line, MD5, were xceptional in that they failed to exhibit silencing of anti-ysozyme B cells, and continued to secrete high levels of transgene-encoded anti-lysozyme IgM into the serum (Fig 4*a*) FACS analysis of spleen cells from both MD5 immunoglobulin- and double-transgenic mice showed that the B cells express high levels of lysozyme-binding IgM and IgD, and that the failure of silencing in the double-transgenic mice was associated with minimal down-regulation of transgene-encoded membrane IgM (Fig 4*b*) The explanation for the lack of membrane IgM down-regulation and B cell silencing in the MD5 line is not known, but may be related to a failure of allelic exclusion between the transgene and the endogenous heavy chain genes, which also appears to be unique to this line

IgM-down-regulation in nontransgenic mice

In spleens from adult nontransgenic mice, three phenotypic populations of B cells have been defined on the basis of IgM and IgD expression[30] B cells expressing little or no IgD and high levels of IgM comprise one subset, designated 'population III' (Fig 5), which is primarily composed of marginal zone B cells in addition to small numbers of immature B cells and Ly1 B cells[31,32] The other two subsets, 'populations I and II' are both relatively long-lived, IgD-positive, recirculating cells found in the follicular mantle zone[31] and are distinguished only on the basis of their membrane IgM levels which vary over a 100-fold range (Fig 5) Low expression of membrane IgM on population I follicular B cells seems to be a post-translational phenomenon, involving increased surface IgM turnover and/or decreased transport to the cell surface[33], but the underlying explanation for such changes in receptor expression is unknown

In the transgenic mice, interaction with a threshold level of self-antigen induced a selective downregulation of membrane IgM and converted B cells with a population II phenotype into population I type cells (Fig 5) The apparent equivalence of the IgD-positive B cells from immunoglobulin- and double-transgenic mice with populations II and I respectively is borne out by the identical expression of non-immunoglobulin B-cell





IG 4 Failure of tolerance and absence of downregulation of membrane IgM in MD5 × ML5 double-transgenic mice *a*, Spontaneous secretion of anti-lysozyme IgM measured in the serum of MD3×ML5 or MD5×ML5 double-transgenic mice or immunoglobulin-transgenic littermates *b*, Spleen cells from MD3×ML5 or MD5×ML5 double-transgenic mice or immunoglobulin-transgenic littermates analysed by FACS for expression of transgene-encoded IgM^a (top), or lysozyme-binding immunoglobulin (bottom) on B220-positive B cells

METHODS Serum anti-HEL IgM antibody was measured as described in Fig 1, except that sheep anti-mouse IgM-alkaline phosphatase was used as the developing reagent FACS analysis was as described previously, IgM^a and B220 were detected with biotinylated RS3 1 and fluoresceinated RA3-6B2 antibodies respectively

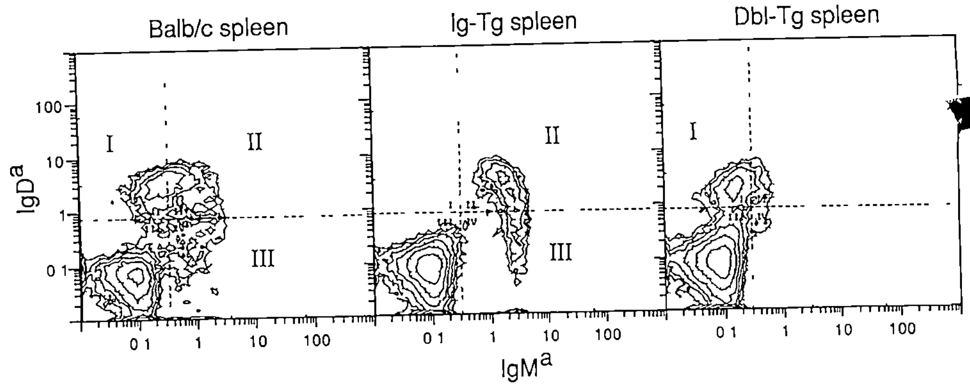


FIG 5 IgD-positive splenic B cells from immunoglobulin and double-transgenic mice are phenotypically equivalent to B cell subpopulations II and I, respectively, in nontransgenic spleen Spleen cells from MD4 immunoglobulin-transgenic or double-transgenic littermates and from an age- and sex-matched BALB/c control were stained for a-allotype IgD and IgM and analysed by FACS, as described in Fig 1 I, II, and III indicate the three predominant B-cell subpopulations defined by relative expression of membrane IgM and IgD[30] To allow consistent use of anti-allotype (transgene-specific) reagents, BALB/c mice were used as nontransgenic controls in preference to C57BL/6 mice, as identical subpopulations are observed in both strains[54 55]

markers[34] and by immunohistological studies showing that the population II and I equivalents in the transgenic mice are indeed both located in the follicular mantle zone (D Y Mason *et al*, in preparation) One interpretation of these findings is that population I and II follicular B cells normally represent a continuum of cells at one extreme, some follicular B cells have high avidity for self antigens (lowest IgM), whereas at the other extreme, B cells exist that do not bind appreciably to any self antigen (highest IgM) The similarity may also hold in functional terms as population I cells with the lowest surface IgM, like double-transgenic B cells, have in fact been found to respond poorly in primary adoptive-transfer antibody responses to foreign antigens[35]

Discussion

Mature follicular B cells expressing transgene-encoded receptors were found to respond either positively to exogenous lysozyme presented on a foreign carrier, or negatively to endogenously synthesized lysozyme These results support and extend earlier evidence for tolerance induction in mature B cells in nontransgenic mice[18-26], and raise the question of how self-reactive B cells are selectively tolerized Because the stage of B-cell development does not seem to be critical for tolerance induction either in the transgenic model or in conventional *in vivo* models[23 25], some other variable must account for the immunogenicity of exogenous lysozyme compared with the tolerogenicity of autologous lysozyme It is tempting to speculate that the factor responsible for determining whether the B cell becomes unresponsive or activated is the absence or presence of second signals derived from helper T cells during the initial contact with antigen, as originally proposed by Bretscher and Cohn[36] Thus, T cell 'help' would be absent during the initial encounter of B cells with above-threshold lysozyme concentrations in the zinc induction experiments, owing to the fact that the low levels of lysozyme expressed in uninduced ML3 mice are still sufficient to render the helper T cell compartment tolerant (S Adelstein *et al*, manuscript in preparation) Similarly, signals from helper T cells would be deficient in the parking experiments during the two days before challenge with HEL-HRBC because the C57BL/6 donor T cells, although not necessarily tolerant to recipient lysozyme, are unable to respond to lysozyme owing to their low-responder genotype[29] In both cases, subsequent encounter with HEL-HRBC would therefore have failed to elicit an antibody response, despite provision of optimal T-cell help, because the B cells had by then become intrinsically unresponsive

The capacity of a threshold concentration of lysozyme corresponding to a receptor occupancy of between 4 5 and 46% to silence mature peripheral B cells provides a straightforward mechanism for preventing the generation of high affinity autoantibodies by hypermutation, and conversely, helps to explain the common occurrence of low affinity autoantibodies in normal individuals The demonstration of a B-cell subset with the phenotype (IgM^{low}, IgD^{hi}) of functionally silenced cells in the peripheral lymphoid tissue of nontransgenic mice lends weight to the possibility that such a mechanism may indeed play a major role in maintaining self-tolerance within the B-cell repertoire

The close relationship between down-regulation of membrane IgM and induction of an unresponsive state in the B cell is reminiscent of ligand-induced receptor downregulation and desensitization in other biological systems[37-40] It is unlikely that the decreased IgM receptor level itself accounts for the unresponsive state of the cell, as membrane IgD is still expressed at high levels and on its own is fully capable of mediating B-cell activation in immunoglobulin-transgenic mice (R Brink *et al*, in preparation) as well as in other models[41,42] Ligand-induced desensitization of IgM and IgD antigen receptors, in the absence of downregulation, has been shown to occur in mature B cells *in vitro*[43] By analogy with other signal-transduction systems[37-40,44,45] biochemical changes affecting subsequent steps in the signalling pathway (reviewed in refs 42, 46, 47), such as phosphorylation or other alterations in membrane immunoglobulin-associated CD3-homologues[48-50], could explain both the selective change in IgM-expression and the unresponsive state of the B cell A detailed understanding of how tolerance mechanisms act on mature peripheral B cells may prove important for controlling pathological antibody responses in allergy and autoimmunity □

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1 Kappler J W Roehm N & Marrack P *Cell* **49**, 273-280 (1987)
2 MacDonald H R *et al* *Nature* **332**, 40-45 (1988)
3 Kisielow P *et al* *Nature* **333**, 742-746 (1988)
4 Sha W C *et al* *Nature* **336**, 73-76 (1988)
5 Pullen A M Marrack P & Kappler J W *Nature* **335**, 796-800 (1988)
6 Lederberg J *Science* **129**, 1649-1653 (1959)
7 Smith C A *et al* *Nature* **337**, 181-184 (1989)
8 Chien Y-H *et al* *Nature* **309**, 322-326 (1984)
9 Ikuta K Ogura T Shimizu A & Honjo T *Proc natn Acad Sci USA* **82**, 7701-7705 (1985)
10 Fink P J *et al* *Nature* **321**, 219-226 (1986)
11 Berek C & Milstein C *Immunol Rev* **105**, 5-26 (1988)
12 French D L Laskov R & Scharff M D *Science* **244**, 1152-1157 (1989)
13 Diamond B & Scharff M D *Proc natn Acad Sci USA* **81**, 5841-5844 (1984)
14 Shlomchik M J Aucoin A H Pisetsky D S & Weigert M G *Proc natn Acad Sci USA* **84**, 9150-9154 (1987)
15 Benjamin D C *et al* *A Rev Immun* **2**, 67-101 (1984)
16 Goodnow C C & Basten A *Sem Immun* (in the press)
17 Lanzavecchia A *Nature* **314**, 537-539 (1985)
18 Chiller J M Habicht G S & Weigle W O *Science* **171**, 813-815 (1971)
19 Katz D H Hamaoka T & Benacerraf B *J exp Med* **136**, 1404-1429 (1972)
20 Weigle W O *Adv Immun* **16**, 61-122 (1973)
21 Howard J G & Mitchison N A *Progr Allergy* **18**, 43-96 (1975)
22 Desaymard C & Waldmann H *Nature* **264**, 780-782 (1976)
23 Metcalf E S Schrater A F & Klinman N R *Immunol Rev* **43**, 143-183 (1979)
24 Scott D W Venkataraman M & Jandinski J J *Immunol Rev* **43**, 241-280 (1979)
25 Parks D E Nelson P A Walker S M & Weigle W O *Ann NY Acad Sci* **392**, 210-222 (1982)
26 Nossal G J V *A Rev Immun* **1**, 33-62 (1983)
27 Goodnow C C *et al* *Nature* **334**, 676-682 (1988)
28 Palmiter R D *et al* *Nature* **300**, 611-615 (1982)
29 Gammon G *et al* *Immunol Rev* **98**, 53-73 (1987)
30 Hardy R R Hayakawa K Parks D R & Herzenberg L A *Nature* **306**, 270-272 (1983)

MacLennan I C M & Gray D *Immunol Rev* **91**, 61-85 (1986)
Herzenberg L A *et al* *Immunol Rev* **93**, 81-102 (1986)
Yuan *D J Immun* **132**, 1566-1570 (1984)
Goodnow C C *et al* *Cold Spring Harbor Symp quant Biol* (in the press)
Zan Bar I Vitetta E S Assisi F & Strober S *J exp Med* **147**, 1374-1394 (1978)
Bretscher P & Cohn M *Science* **163**, 1042-1049 (1970)
Hari J & Roth R A *J biol Chem* **262**, 15341-(1987)
Gill G N *et al* *Cold Spring Harbor Symp quant Biol* **53**, 467-476 (1988)
Williams L T Escobedo J A Keating, M T & Coughlin S R *Cold Spring Harbor Symp quant Biol* **53**, 455-465 (1988)
Sherr C J Rettenmier C W & Roussel M F *Cold Spring Harbor Symp quant Biol* **53**, 521-530 (1988)
Goroff D K Stall A Mond J J & Finkelman F D *J Immun* **136**, 2382-2392 (1986)
Cambier J C *et al* *Immunol Rev* **95**, 37-57 (1987)
Cambier J C *et al* *Proc natl Acad Sci USA* **85**, 6493-6497 (1988)
Lefkovitz R J *et al* *Cold Spring Harbor Symp quant Biol* **53**, 507-514 (1988)
45 Stryer L *Cold Spring Harbor Symp quant Biol* **53**, 283-294 (1988)
46 Mueller, D L Jenkins M K & Schwartz R H *A Rev Immun* **7**, 445-480 (1989)
47 Defranco A L Gold M R & Jakway J P *Immunol Rev* **95**, 161-176 (1987)
48 Hombach J *et al* *EMBO J* **7**, 3451-3456 (1988)
49 Sakaguchi N *et al* *EMBO J* **7**, 3457-3464 (1988)
50 Reth M *Nature* **338**, 383-384 (1989)
51 Schuppel R Wilke J & Weiler E *Eur J Immun* **17**, 739-741 (1987)
52 Smith Gill S J Lavoie T B & Mainhart C R *J Immun* **133**, 384-393 (1984)
53 Sedgwick J D & Holt P G *J immunol Meth* **57**, 301-311 (1983)
54 Hardy R R Hayakawa K Haaijman J & Herzenberg L A *Nature* **297**, 589-591 (1982)
55 Herzenberg L A *et al* *Nature* **329**, 71-73 (1987)

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The guanosine binding site of the *Tetrahymena* ribozyme

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he self-splicing Group I introns have a highly pecific binding site for the substrate guanosine lutant versions of the *Tetrahymena* ribozyme ave been used in combination with guanosine nalogues to identify the nucleotide in the ribozyme hat is primarily responsible for recognition of the uanine base

NE of the substrates of the class of ribozymes known as the roup I introns is the nucleoside guanosine, an understanding f how this substrate is bound is essential to an understanding f the mechanism of catalysis of these RNA enzymes All reactions performed by Group I introns seem to involve the binding ither of guanosine or an internal G residue The first step of elf-splicing requires the specific binding of free guanosine, hich becomes covalently added to the 5′ end of the intron after leavage of the 5′ intron–exon junction[1] Group I introns invariably end with a 3′ terminal G residue (for a review, see ref 2), hich is required for efficient cleavage and ligation at the 3′ tron–exon junction[3] Reactions that mimic circularization of e excised intron also require guanosine[4]

It is clear that the Group I introns contain a well-defined uanosine-binding site Bass and Cech[5 6] used guanosine nalogues to show that both the ribose and guanine moieties of uanosine are specifically recognized by the *Tetrahymena* intron ere we present evidence that a phylogenetically invariant uanine residue in the highly conserved stem P7 in the catalytic ore of the intron is the part of the guanosine binding site that nteracts with and recognizes the guanine base

ase-paired stem constrains binding-site location

he site that binds the 3′-terminal G residue of the *Tetrahymena* ibozyme must be physically close to the site that binds the 3′ ntron–exon junction We therefore first attempted to clarify the ature of the 3′ intron–exon junction binding site A part of the ntron called the internal guide sequence (IGS) is known to ind the 5′ exon, formation of a base-paired stem (P10) between he 3′ exon and the IGS has been proposed to align the two xons for ligation (ref 7, and Fig 1) The P10 pairing is not resent in all Group I introns, however, and can be disrupted in the *Tetrahymena* intron without affecting splicing[8] These data indicate that the 3′ intron-exon junction could be recognized by redundant mechanisms A probable candidate for such an additional interaction is the recently identified P9 0 pairing between the two nucleotides immediately preceding the 3′ terminal G residue, and two nucleotides in the segment (J7-9) connecting base-paired stems P7 and P9 (F Michel, P Netter, M Xu and D Shub, manuscript submitted) The P9 0 pairing was identified by sequence comparison analysis, and is widespread although not universal in the Group I introns

By mutationally disrupting the P9 0 and P10 putative pairings, both separately and together (Fig 1 and Table 1), we found that both contribute to binding the 3′ intron–exon junction in the *Tetrahymena* intron None of these mutations had marked effects on the rate of 5′ cleavage Mutants with only the P9 0 or the P10 pairing disrupted had relatively minor effects on 3′ splicing By contrast, when both P9 0 and P10 pairing were disrupted, 3′ splicing was strongly affected, these introns accumulate 5′ exon and intron-3′ exon during splicing Restoring either the P9 0 or P10 pairing by compensatory mutations invariably alleviates the defect in 3′ cleavage and ligation Thus,

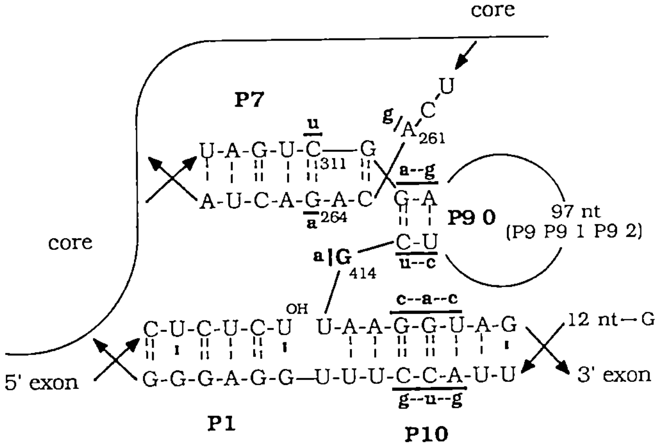

FIG 1 Secondary structure elements surrounding the 3′ terminal G (position 414) of the *Tetrahymena* intron P1, P10, P7 and P9 0 are base-paired segments (See refs 9-11 and 23 for nomenclature and a more complete secondary structure model of the intron) The situation depicted is just before the ligation step Mutations introduced in this work are indicated in bold, lower case letters next to the nucleotides that were substituted nt, Nucleotide

binding of the 3′ intron–exon junction is ensured by base pairing of the nucleotides immediately flanking the terminal G residue to two distinct regions of the *Tetrahymena* intron. These two pairings strongly constrain the possible locations of the guanosine binding site.

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Defective recognition of the 3′ terminal G

We searched for an intron residue that might be involved in recognition of the 3′ terminal G by screening mutants for the loss of ability to distinguish between a G and an A residue at the 3′ end of the intron. Changing the 3′ terminal G residue of the *Tetrahymena* intron (position 414) to a U or C residue results in normal cleavage at the 5′ intron–exon junction, but greatly reduces the rates of 3′ cleavage and ligation[3]. Under the same splicing conditions, we found that changing the 3′ terminal G to an A residue led to delayed (but still accurate) 3′ splicing.

Because all Group I introns end in a G residue we surmised that any nucleotide involved in recognition of this 3′ terminal G was probably one of the very few residues that are conserved in all Group I introns. There are only two such invariant nucleotides close to P9.0 in the secondary structure of the *Tetrahymena* intron[2,9–11]. These are residue A at position 261 (A261) in the joining segment J6/7, which we mutated to G (mutation A261→G), and residue G264, which is part of the very highly conserved paired stem P7: the G264·C311 pair was mutated to A·U (mutation A·U).

Both the A261→G and the A·U mutations greatly reduced the rate of 5′ cleavage, but at high concentrations of GTP, cleavage was efficient enough to allow study of 3′ splicing. In an A261→G context, the rate of 3′ splicing was further decreased when the 3′ terminal G residue was mutated to A (Fig. 2*a*, *c*), showing that position 261 is not involved in recognition of the 3′ terminal G. The A·U mutation, by contrast, greatly decreased the rate of 3′ cleavage, but there was only a slight further decrease on changing the 3′ terminal G residue to A (Fig. 2*b*, *d*). Either recognition of the 3′ terminal nucleotide was no longer rate-limiting for the A·U introns, or they failed to distinguish G from A.

A guanosine analogue restores 5′ cleavage

If the same binding site is used for both the 3′ terminal G residue and the free guanosine used in 5′ cleavage, the A·U mutant should have a greatly reduced affinity for free guanosine. Although we were not able to measure guanosine binding (dissociation constant, K_d) directly, we were able to measure the Michaelis constant K_m (depending on the precise reaction kinetics, K_m need not equal K_d), and we found that the K_m for 5′ cleavage of the A·U intron with guanosine is 3.0 mM; this is two orders of magnitude higher than the K_m for 5′ cleavage of the wild-type intron, which is 0.024 mM (Fig. 3*a*, Table 2). I addition, the maximum initial rate of the reaction is much re duced (0.0034 min^{-1} instead of 0.29 min^{-1}), again, the catalyti constant K_{cat} need not reflect the chemical step of catalysis, bu the simplest interpretation of these results is that the guanosin is not only poorly bound, but incorrectly orientated.

A model of possible hydrogen-bonding interactions betwee guanosine and the G264·C311 base pair is shown in Fig. 4. I this model, the G264→A mutation would lead to a steric clas between two hydrogen-bond donors, resulting in both decrease affinity and incorrect positioning. To test this model, we synthesized the guanosine analogue 2-aminopurine ribonucleosid (2AP), which differs from guanosine in its distribution of hydrogen-bond donors and acceptors. The interactions proposed fo the binding of 2AP to the A of an A·U base pair and for th binding of G to G·C are isosteric (Fig. 4). When we used 2A in splicing reactions, we found that wild-type introns preferre G to 2AP, but A·U introns preferred 2AP to G (Table 2, Fig. 3*b*). The ability of the A·U mutant to perform 5′ cleavage wa fully restored at high concentrations of 2AP, and the K_m of th A·U mutant with 2AP (two hydrogen bonds, $K_m = 0.43$ mM) is

TABLE 1 Effect of disruption of the P9.0 and P10 pairings on exon ligati

P10	P9.0			
	Wild type	5′-disrupted	3′-disrupted	Restore
Wild type	0.91	0.42	0.60	0.85
5′-disrupted	0.74	0.05	0.011	0.20
3′-disrupted	0.92	0.10	0.31	0.88
Restored	0.96	0.59	0.73	0.94

Mutations introduced to disrupt the P9.0 and P10 pairings are shown Fig. 1. Numbers shown are the fraction of 5′ cleaved exon that has beco ligated to 3′ exon. Reactions were for 60 s, the fraction of reacted precurs varied from 22–87% depending on the mutant examined. Missplicing w minimal for all mutants, as the only band of significant intensity inbetwe linear intron (~400 nucleotides (nt)) and 5′ exon (57 nt) was the ba generated by ligated exons (101 nt). The only exception was the muta disrupted through mutation of the 3′ branches of both P9.0 and P10: a ba migrating to a position corresponding to 64 nt was observed that amount to ~20% of the sum of ligated exons and 5′ exon. Mutations were co structed by casette mutagenesis as previously described[19]. Transcriptic gel purification of [^{32}P]GTP-labelled full-length transcripts, splicing conditio (5 mM $MgCl_2$, 200 mM $NH_4C_2H_3O_2$, 20 mM Tris buffer pH 7.5, 0.02% SD 30 °C, 20-min preincubation, 2 mM GTP) and gel electrophoresis were esse tially as described previously[3]. The radioactivity in electrophoretic banc was determined by direct scanning with a Betagen counter (Walthar Massachusetts) and converted to molar amounts by correcting for t number of G residues.

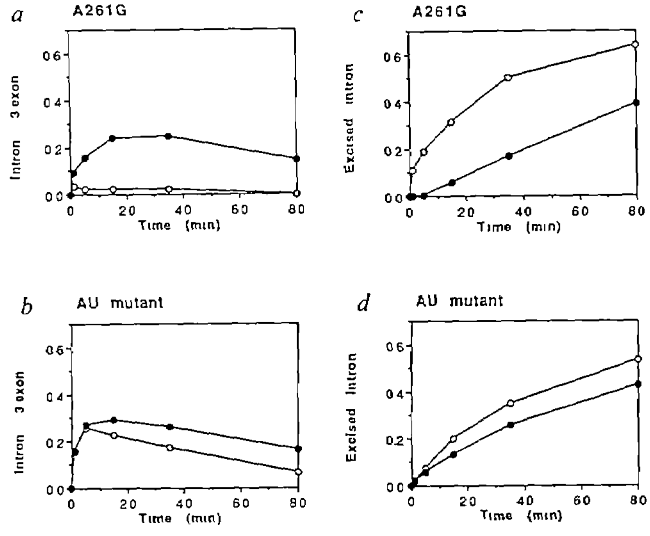


FIG. 2 Ability of two mutant versions of the intron to distinguish between 3′ terminal G (○) and 3′ terminal A (●). *a*, *b*, Accumulation of the intron–3′ exon intermediate, expressed as fraction of total intron. *a*, A261G mutant. This intermediate does not accumulate with a 3′ terminal G, but does with a 3′ terminal A. *b*, AU mutant. This intermediate accumulates with similar kinetics whether or not the 3′ terminal residue is A or G. *c*, *d*, Accumulation of excised intron after 3′ cleavage. *c*, A261G mutant. Accumulation is delayed when the 3′ terminal residue is A rather than G. *d*, AU mutant. Product accumulates with similar kinetics with either 3′ terminal A or G.
METHODS. Splicing was carried out in 15 mM $MgCl_2$, 40 mM Tris buffer pH 7.5, 2 mM spermidine, 5 mM DTT at 37 °C. Reactions were started by addition of GTP to a final concentration of 2 mM (<10% of precursor RNA had reacted after 80 min in controls without GTP).

ver than that of either the A U mutant with guanosine (one drogen bond, steric clash, $K_m = 3\ 0$ mM) or the wild-type ron with 2AP (one hydrogen bond, no steric clash, K_m = mM)

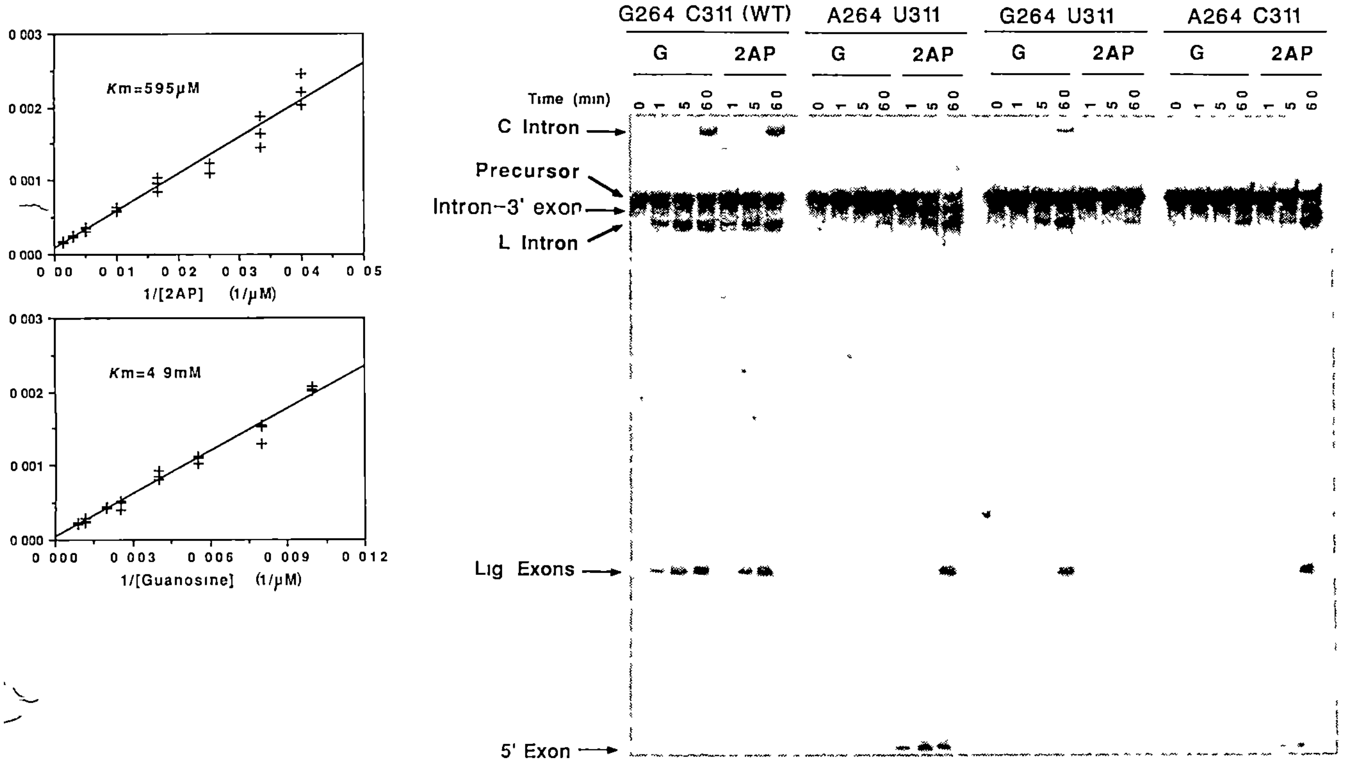
We used single mutants to show that the G residue of the :64 C311 base pair is responsible for discriminating between anosine and 2AP A G C-to-G U mutation still allowed some icing with guanosine, but almost no splicing was detectable :h 2AP (Fig 3*b*) By contrast, a G C to A C mutation blocked icing with guanosine, but allowed cleavage by 2AP

e A·U mutation affects inhibition by arginine

e amino acid arginine inhibits self-splicing of the *Tetrahymena* ron by competing with guanosine[12] Yarus[12] proposed that : guanidino group of arginine can substitute for the two donor oups of guanine (Fig 4) If so, splicing of the A U mutant ould not be inhibited by arginine, for the same reason that s mutant binds guanosine so poorly We found that splicing the wild-type intron was strikingly inhibited by 10 mM ginine, whereas no effect was seen on the activity of the A U mutant (Fig 3*c*) Citrulline is an analogue of arginine with a substitution in the guanidino group which should enable it to bind to the A U mutant intron (Fig 4) Citrulline was reported to be only a very weak inhibitor of splicing[13] and we observed only slight inhibition of splicing of the wild-type intron at 300 mM citrulline But splicing of the A U mutant was significantly more inhibited by citrulline (Fig 3*c*), as predicted by the model for arginine binding

2-aminopurine in exon ligation

If there is one binding site for both free guanosine and the 3′ terminal G of the *Tetrahymena* intron, it should be possible to restore the A U mutant to full 3′ splicing activity by replacing the 3′ terminal G residue by 2AP Unfortunately, direct replacement of a nucleotide by an analogue in a long polynucleotide chain is not feasible at present The exons to be ligated, however, need not be covalently linked to the intron and can be quite short[14] We therefore synthesized two oligoribonucleotides that could be aligned on the IGS of the *Tetrahymena* intron One oligonucleotide represented the 5′ exon, the other represented



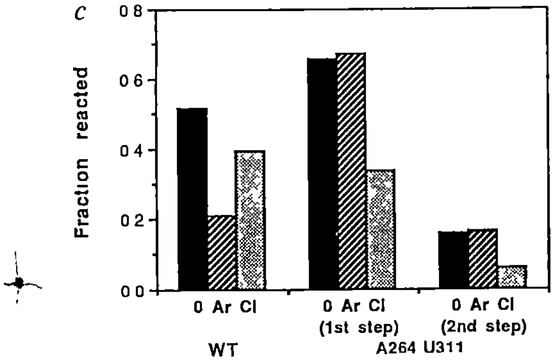


FIG 3 Splicing activity of mutant transcripts with guanosine and guanosine analogues *a*, Kinetics of 5′ cleavage of the A U mutant with guanosine (lower panel) and 2AP (upper panel) V_0 is the initial rate of the reaction, expressed in counts of released product (free 5′ exon plus 5′ exon component of ligated exons) *b*, Autoradiograph of a 5% acrylamide–8 M urea gel showing splicing products generated by incubation of wild-type (WT) and mutant transcripts with guanosine and 2-aminopurine at 1 5 mM Lig, Ligated *c*, Inhibition of splicing of wild-type and mutant transcripts by arginine and citrulline Reactions were started by addition of 3 μM guanosine to wild-type intron or 50 μM 2AP to A U mutant intron together with 10 mM arginine or 300 mM citrulline Histograms show the extent of reaction at 30 min, normalized to the final amount of precursor reacted at 2 h For the A U mutant, 1st step corresponds to 5′ cleavage (intron-3′ exon in addition to excised intron), and 2nd step corresponds to both 5′ and to 3′ cleavage (excised intron only)

METHODS For K_m and V determinations, triplicate measurements were made at eight substrate concentrations RNA and ribonucleoside were separately pre-equilibrated in buffer at 30 °C for 10–30 min Reactions were started by addition of ribonucleoside and stopped by addition of an equal volume of 90% formamide, 25 mM EDTA after 1 min (WT, mutant with 2AP) or 2 h (mutant with guanosine) RNA concentrations ranged from 45–90 nM Other methods as in Table 1 he 3′ splice site The 3′ splice site oligonucleotide began with G residue, this G is equivalent to the 3′-terminal G of the ormal intron, and is cleaved off during the ligation reaction The two oligonucleotides were efficiently ligated by a shortened form of the *Tetrahymena* intron lacking both normal splice sites Fig 5) We took advantage of the fact that this reaction will un in reverse to generate a modified 3′-exon oligonucleotide eginning with 2AP an oligonucleotide representing ligated xons was cleaved by the A U mutant intron in the presence of ree 2AP

We could then compare the ability of the 3′-exon oligonucleoide beginning with G and its 2AP-substituted version to serve s 3′ splice sites As expected, the A U mutant intron was unable o ligate a 3′ exon beginning with G, but was able to ligate a 3′ xon beginning with 2AP (Fig 5*c*) By contrast, the 2AP oligonucleotide was not used by the wild-type intron Therefore, in reaction that mimics 3′ cleavage and exon ligation, the G C nd A U versions of the *Tetrahymena* intron show the same preferences for guanosine and 2AP as they do in 5′ cleavage This, together with the fact that the A U mutant distinguishes poorly between G and A at the 3′ intron–exon junction, shows hat free guanosine and the 3′ terminal G are not only recognized by the same nucleotide of the intron but most probably bind it by means of the same type of G G base pair

Discussion

The guanosine-to-G C base triple postulated in Fig 4 readily accounts for data reported by Bass and Cech[5] concerning the ability of the *Tetrahymena* intron to use guanosine analogues Both 1-methylguanosine and *N*-2-methylguanosine are poor ubstrates for splicing, as expected from the involvement of positions 1 and 2 in hydrogen bonding By contrast, positions 7 and 8, which are not involved, can be substituted without effect We have attempted to determine the role of position 6 by comparing the abilities of 2AP and 2,6-diaminopurine ribonucleosides to participate in 5′-cleavage if 06 of guanosine is nvolved in hydrogen bonding, replacing it by a donor group would presumably create a steric clash, and therefore be highly detrimental Our experiments (Table 2), however, show that 2,6-diaminopurine is nearly as effective as 2AP at saturating concentrations, whether wild-type or mutant introns are involved It is possible that only two hydrogen bonds are formed between the bound guanine base and the intron, with stacking nteractions also contributing to the high affinity and highly specific recognition of guanosine The specificity of ribonuclease T1 towards guanine nucleotides rests similarly on the base stacking with a tyrosine residue, in addition to the formation of two hydrogen bonds with the main chain of this protein enzyme[15] Lower stacking energies due to the loss of 06 might account for the elevated K_m of the A U mutant for 2AP (and the even higher K_m for 2,6-diaminopurine), compared with that of wild type for guanosine

It has been argued that Group I introns possess two G-binding sites[16] This suggestion stemmed from the G-exchange reactions in which a 3′-OH group of a 3′ terminal G attacks the phosphate immediately 3′ to a G residue[17,18] But our results show that there cannot be two completely separate binding sites, because the same mutation affects the G-residue requirement for both 5′ and 3′ splicing A further argument in favour of a single G-binding site in Group I introns is that all cyclization reactions involving mismatched combinations of nucleotides and binding site are subject to competition by the better-matched free nucleoside For instance, excised intron molecules carrying the A U mutation circularize slowly, and this reaction is inhibited by 2AP but not by guanosine

The interaction between guanosine and the highly conserved G C base pair in the core of the ribozyme constitutes our first glimpse of how an RNA enzyme binds a mononucleotide substrate Similar experiments with guanosine analogues and mutant introns should identify the bases involved in stacking interactions and the groups recognizing the ribose □

TABLE 2 Splicing of wild-type and mutant introns with guanosine and analogues

RNA	Purine nucleoside	K_m (mM)	V (min^{-1})	V/K_m (M^{-1} min^{-1})
Wild-type	Guanosine	0 027	0 34	12,500
		0 021	0 24	11,500
	2-Aminopurine	1 09	0 17	156
	2,6-Diaminopurine	2 2	0 14	63
		1 4	0 17	119
Mutant	Guanosine	2 4	0 0019	0 81
		3 7	0 0049	1 33
	2-Aminopurine	0 59	0 68	1,140
		0 28	0 54	1,930
	2,6-Diaminopurine	3 6	0 26	73
		1 8	0 25	139

Velocity of enzyme-catalysed reaction at infinite concentration of substrate (V) and K_m values were calculated from plots as in Fig 3*a* V is expressed as the fraction of precursor RNA converted per min, corrected for the maximum extent of reaction Reactions were for 60 s, except for mutant transcripts with guanosine (2 h) Different values for the same transcript and analogue correspond to different RNA preparations Multiple determinations with the same RNA preparation were averaged Splicing conditions were as in Table 1, and kinetic analysis as in Fig 3 Synthesis of 2-aminopurine thioguanosine (Sigma) was directly reduced to 2-aminopurine ribonucleoside with Raney nickel[20] The reaction was followed by ultraviolet spectroscopy absorption at 340 nm decreased and that at 306 nm increased A fine yellow precipitate was recovered and analysed The ultraviolet spectrum was identical to that published by Fox *et al*[20], and the NMR spectrum clearly indicated that the starting thioguanosine had been quantitatively (>99%) reduced Synthesis of 2,6-diaminopurine riboside (an initial sample was a gift of L Orgel) guanosine was quantitatively converted to 2,6-diaminopurine riboside in a silylation-amination procedure using hexamethyldisilazane and ammonia in the presence of a Lewis acid, trimethylsilyl-trifluoromethanesulphonate[21] The product was recrystallized three times from boiling water to yield fine white crystals Its NMR spectrum was identical to that found by Muraoka[22] and showed no evidence of contamination by guanosine

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1 Cech T R *et al* *Cell* **27**, 487–496 (1981)
2 Cech T R *Gene* **73**, 259–271 (1988)
3 Price J V & Cech T R *Genes Dev* **2** 1439–1447 (1988)
4 Tanner N K & Cech T R *Biochemistry* **26**, 3330–3340 (1987)
5 Bass B L & Cech T R *Nature* **308**, 820–826 (1984)
6 Bass B L & Cech T R *Biochemistry* **25**, 4473–4477 (1986)
7 Davies R W *et al* *Nature* **300**, 719–724 (1982)
8 Been M D & Cech T R *Nucleic Acids Res* **13**, 8389–8408 (1985)
9 Michel F & Dujon B *EMBO J* **2**, 33–38 (1983)
10 Waring, R B *et al* *J molec Biol* **167**, 595–605 (1983)
11 Cech T R *et al* *Proc natn Acad Sci USA* **80**, 3903–3907 (1983)
12 Yarus M *Science* **240**, 1751–1758 (1988)
13 Yarus M *Biochemistry* **28**, 980–988 (1989)
14 Kay P S & Inoue T *Nature* **327**, 343–346 (1987)
15 Heinemann O & Saenger W *Nature* **299**, 27–31 (1982)
16 Kay P S *et al* *EMBO J* **7**, 3531–3537 (1988)
17 Inoue T *et al* *J molec Biol* **189**, 143–165 (1986)
18 Van der Horst G & Tabak H F *EMBO J* **6**, 2139–2144 (1987)
19 Doudna J A *et al* *Cold Spring Harb Symp quant Biol* **52**, 173–180 (1987)
20 Fox J J *et al* *J Am Chem Soc* **80**, 1669–1675 (1958)
21 Vorbruggen H & Krolikiewicz K *Liebigs Ann Chem* **4**, 745–761 (1976)
22 Muraoka M *Chem pharmacol Bull* **29**, 3449–3454 (1981)
23 Burke J M *et al* *Nucleic Acids Res* **15**, 7217–7221 (1987)
24 Szostak J W *Nature* **322**, 83–86 (1986)
25 Milligan J F *et al* *Nucleic Acids Res* **15**, 8783–8798 (1987)

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The barrier to recombination between *Escherichia coli* and *Salmonella typhimurium* is disrupted in mismatch-repair mutants

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The requirement for DNA sequence homology in generalized genetic recombination is greatly relaxed in bacterial *mutL*, *mutS* and *mutH* mutants deficient in mismatch repair In such mutants, intergeneric recombination occurs efficiently between *Escherichia coli* and *Salmonella typhimurium*, which are ~20% divergent in DNA sequence This finding has implications for speciation, for regulating recombination between diverged repeated sequences, and for hitherto difficult interspecies hybridizations

GENERALIZED genetic recombination provides a means for the transfer or exchange of genetic information between homologous regions of DNA, as well as for the repair of DNA damage The fidelity of recombination depends on the precise formation of a hybrid or heteroduplex joint consisting of two complementary DNA strands, one contributed by each of the recombining molecules [1-4] The hybrid joint is a key structure for aligning parental sequences in genetic recombination Presumably because of the need to maintain chromosomal integrity, recombination *in vivo* requires nearly perfect homology and is abolished by as little as 10-20% sequence divergence[5,6] But heteroduplex DNA formation catalysed *in vitro* by the *E coli* RecA protein tolerates up to 30% sequence mismatches[7] Several lines of evidence indicate that the strand exchange reaction is edited by mismatch repair proteins supposed to abort or inactivate heteroduplex intermediates containing mismatched base pairs[8,9] Karimova *et al* [10] were first to propose that recombination between highly diverged sequences might be differentially inhibited by the mismatch repair system (owing to overlapping excision tracts[11-13]), this idea has since been independently proposed by others[8,9 14,15] Studies on recombination and mismatch repair in *Streptococcus pneumoniae*[16,17] and *E coli*[14,18] indicate that the marker effects on the efficiency of homologous recombination are often caused by the abortion of heteroduplex intermediates with one or few mismatches[8] Thus, the mismatch repair system might prevent recombination between partially homologous sequences and provide a functional barrier to interspecies recombination[8,9] We have now tested the prediction that mutants defective in mismatch repair can become proficient in interspecies recombination by using members of two related bacterial genera, *E coli* and *Salmonella typhimurium*, which are about 80% homologous in DNA sequence[19] Mutations in the *mutL*, *mutS* or *mutH* mutator genes inactivate methyl-directed mismatch repair of errors in DNA replication[20-22], we show that these mutations can increase intergeneric recombination in conjugational and transductional crosses by up to 1,000-fold Hence, mismatch recognition and the mismatch repair system can act as a barrier to recombination between DNAs of different species

Conjugation

Conjugation between *E coli* Hfr donors and *S typhimurium* I recipients deficient in DNA restriction (see Table 1 for stra description and the legend of Table 2 for the protocol) w conducted for 40 min on the surface of nutrient agar plate Mating was interrupted and the bacteria were replated to sele for xyl^+ or met^+ *S typhimurium* recombinants under conditio that prevented the growth of the *E coli* donor cells When th recipient cells were deficient in methyl-directed mismatch repa that is, *mutH*, *mutL*, *mutS* or *mutU*, recombinants we recovered much more frequently than when the recipients we mut^+ (Table 2) The increase in the formation of recombinan is clearly *recA* dependent (The relatively weak effect of *mut* (leading to a lack of helicase II function)[23-25] was not due the complementation by the *E coli* $mutU^+$ gene—Table 2 The co-inheritance of *xyl* and *met* markers, separated by ~13% of the bacterial genome, indicates that large fragments of th *Escherichia* genome can be integrated into the *Salmonell* recipient genome, although less frequently than in the homo specific *E coli* cross (Table 3)

Perhaps the simplest interpretation of these results is tha intergeneric recombinants arise through gene replacement, th

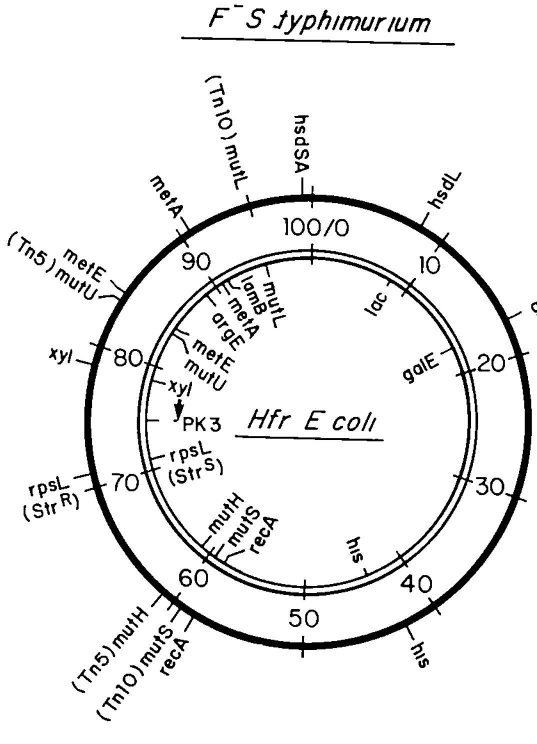

FIG 1 Genetic maps of *E coli* Hfr*PK3* and *S typhimurium LT2* F⁻ used in the experiments described here The origin of transfer of the Hfr *PK3* is indicated by an arrow (from refs 54-56)

TABLE 1 Genotypes and origins of bacterial strains

	Reference
Escherichia coli	
F^- AB1157 *thr1 leu6 proA2 his4 thi1 argE3 lacY1 galK2 ara14 xyl5 mtl1 tsx33 str31 supE44*	54
Hfr PK3 *thr1 leuB6 thi1 lacY1 azi15 tonA21 supE44*	55
Hfr PK3 *mutU (uvr D260 Tn5)*	This work[*]
Salmonella typhimurium	
F^- SL4213 *galE496 metA22 metE551 rpsL120 xyl404* (Fels2) *H1b nml H2-enx ilv452 hsdSA29 hsdL6*	56
F^- SL4213 *mutH* Tn*5*	57
F^- SL4213 *mutL* Tn*10*	57
F^- SL4213 *mutS* Tn*10*	57
F^- SL4213 *mutU* Tn*5*	57
F^- SL4213 *mutH* Tn*5 recA1*	This work[†]
F^- SL4213 *mutL* Tn*10 recA1*	This work[†]
F^- SL4213 *mutS* Tn*10 recA1*	This work[†]

[*] Made by P1 transduction[58] from *E coli* GW7303 (AB1157 *uvrD260* Tn*5*), selection for kanamycin resistance (40 μg ml^{-1}) and screening for mutator and UV-sensitive phenotype

[†] Made by P22 *int3* Hft transduction from *S typhimurium* TT13479 (*recA1 srl203* Tn*10d-Cam*—which confers chloramphenicol resistance)[34], selection for chloramphenicol resistance (10 μg ml^{-1}) and screening for sensitivity to ultraviolet

usual outcome of intraspecies mating It is possible for phenotypic recombinants to arise through other mechanisms however, such as (1) recA-dependent circularization of the incoming DNA, perhaps using blocks of homology such as those found in ribosomal RNA *rrn* genes, and perpetuation of the circle as an autonomous plasmid (because the conjugated region includes the *E coli oriC* origin of replication) and (2) formation of interspecies duplications involving recombination between sequences common to both organisms[26–29]

Phenotypic recombinants resulting from plasmid or duplication formation should be less stable than recombinants resulting from gene replacement To test for the stability of the xyl^+ recombinants, colonies from each cross were streaked on indicator plates Sectoring of the originally red colonies to white (the phenotype of the recipient before conjugation) or to dark red (the phenotype expected if multiple copies of the gene are present) indicates an unstable phenotypic recombinant The fractions of stable recombinants are given in Table 3 and range from 1 of 35 for *mutS* recipients to 19 of 35 for *mutU* recipients and 33 of 34 for mut^+ recipients If the recombinant frequencies in Table 2 are corrected for the fraction of unstable recombinants, the frequencies of stable recombinants from mut^- recipients range from 2×10^{-5} (*mutU*) to 7×10^{-4} (*mutL*), an increase of one to three orders of magnitude over the frequencies obtained with mut^+ recipients The highest frequencies of recombinants came from *mutL* and *mutS* recipients which could be a result of the involvement of the MutL and MutS proteins in the first step of the mismatch repair process[30] For example, it could be that the binding of these mismatch repair proteins to mismatch-containing DNA is sufficient to disrupt recombination even under conditions of complete DNA methylation Alternatively, it could be that the incoming strands are undermethylated (perhaps newly synthesized)[31] or that formation of partially homologous heteroduplex DNA involves extensive DNA synthesis in the flanking regions creating hemimethylated regions which would be substrates for normal mismatch repair activity

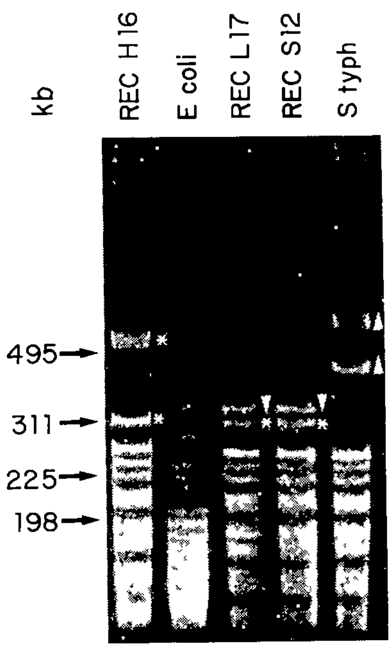

FIG 2 Restriction pattern of intergeneric recombinants from *E coli* Hfr and *S typhimurium* F^- crosses Three stable xyl^+ met^+ recombinants (REC) were selected from crosses between *E coli* Hfr *PK3* and *S typhimurium* F^- recipients which were either *mutH* (for REC H16), *mutL* (for REC L17) or *mutS* (for REC S12) For REC L17, the original *S typhimurium mutL* Tn*10* locus was replaced by the $mutL^+$ locus from *E coli*, as shown genetically in Table 3 △, Parental *S typhimurium* F^- DNA fragments lost in recombinants, ▽, DNA fragment from recombinant genomes co-migrating with a parental *E coli* Hfr donor DNA fragment, *, Non-parental DNA fragments Fragment size markers are yeast *S cerevisiae* no 334 chromosomes IX, III, VI and I Kb, kilobases

METHODS Bacteria (10 ml) were grown in Luria Bertani medium to 1 5–2 × 10^8 cells ml^{-1}, chloramphenicol was added to 180 μg ml^{-1} and incubation was continued for 1 h The bacteria were chilled, pelleted at 4 °C, resuspended in 10 ml solution I (10 mM Tris-HCl, pH 7 6, 1 M NaCl), pelleted again, and resuspended in 1 5 ml solution I They were warmed to 40 °C and an equal volume of 1 5% low-melting-point agarose was added The mixture was put in Tygon tubing, allowed to solidify, and extruded into an equal volume of lysis solution I (6 mM Tris-HCl, pH 7 6, 1 M NaCl, 100 mM EDTA, pH 7 5, 0 5% Brij 35, 0 2% deoxycholate, 0 5% sarcosyl, 1 mg ml^{-1} lysozyme, 0 2 mg ml^{-1} bovine pancreatic RNase) and incubated overnight at 37 °C with gentle shaking (With these amounts, about 25 plugs with 0 5 μg DNA per plug can be made) The plugs were then put in an equal volume of lysis solution II (0 5 M EDTA, pH 9 3, 1% lauroyl sarcosine, 1 mg ml^{-1} proteinase K) and incubated for 2 days at 50 °C The lysis solution II was replaced and the plugs were stored at 4 °C until used Plugs were then put in 15 ml TAFE buffer (10 mM Tris buffer, 4 37 mM glacial acetic acid, 0 5 mM free-acid EDTA) at room temperature for 1–2 h with gentle inversion of the tubes They were then put in new TAFE buffer with 5 mM phenylmethylsulphonyl fluoride (PMSF) and kept for another hour in the same conditions and then successively in 7 ml TE (10 mM Tris-HCl, pH 7 6, 1 mM EDTA, pH8) for 15 min with gentle inversion of the tubes and in *Spe*I-restriction buffer containing 2 mM PMSF for 10 min at 37 °C Ten units of *Spe*I enzyme (New England Biolabs) were then added to each plug, which was incubated overnight at 37 °C The plugs were then put in transverse alternating-field electrophoresis (TAFE) buffer and then in wells of 1 3% agarose gel and sealed with 1 5% agarose in TE Electrophoresis in a Beckman Gene Line TAFE system was at 220 V with two switch pulses of 25 s each for 21 h at 14 °C The gel was then stained in 0 5 μg ml^{-1} ethidium bromide, destained, and illuminated with ultraviolet light The low-melting-point agarose, the agarose and the TAFE buffer were Beckman Gene Line products The experiment was done in the Beckman Instruments Spinco Division in Palo Alto

TABLE 2 Conjugational crosses between *Escherichia coli* and *Salmonella typhimurium*

E coli Hfr donor	*S typhimurium* F$^-$ recipient	*E coli* F$^-$ recipient	*xyl*$^+$ per Hfr	*metE*$^+$*metA*$^+$ per Hfr
mut$^+$	*mut*$^+$		9×10^{-7}	2×10^{-6}
mut$^+$	*mutS*		2×10^{-3}	2×10^{-3}
mut$^+$	*mutL*		3×10^{-3}	2×10^{-3}
mut$^+$	*mutH*		6×10^{-5}	2×10^{-4}
mut$^+$	*mutU*		5×10^{-5}	9×10^{-6}
mut$^+$	*mut*$^+$ *recA*		7×10^{-8}	
mut$^+$	*mutS recA*		$<2\times10^{-8}$	
mut$^+$	*mutL recA*		3×10^{-7}	
mut$^+$	*mutH recA*		4×10^{-8}	
mutU	*mutU*		3×10^{-5}	
mutU	*mutL*		3×10^{-3}	
mut$^+$		*mut*$^+$	3×10^{-1}	
mutU		*mut*$^+$	2×10^{-1}	

Heterospecific crosses involved *E coli* Hfr PK3 or PK3 *mutU* and *S typhimurium* F$^-$ SL4213 *mut*$^+$, *mutH*, *mutL*, *mutS*, *mutU* or F$^-$ SL4213 (*mut*$^+$ or *mut*) *recA*1 (see Table 1) Homospecific crosses involved *E coli* Hfr PK3 or PK3 *mutU* and *E coli* F$^-$ AB1157 Log-phase bacteria were mixed in a 1 1 (Hfr F$^-$) ratio and immediately deposited on a sterile 0 45-μm pore size filter (Schleicher and Schuel) which was incubated on prewarmed, rich-medium agar After 40 min at 37 °C, the conjugants were resuspended in 10^{-2} M $MgSO_4$ and viciously separated by vortexing The exconjugants were then plated on M63 synthetic medium[58] either lacking methionine to select for *met*$^+$ recombinants or containing xylose as the sole carbon source to select for *xyl*$^+$ recombinants The Hfr donor cells were doubly counterselected by 25 μg ml^{-1} streptomycin and, in the case of intergeneric conjugation, by withholding threonine and leucine Amino acids were added to a final concentration of 100 μg ml^{-1}, thiamine to 30 μg ml^{-1} and xylose to 0 4% Because of late appearance of intergeneric recombinants from *mut*$^+$ and *mutU* crosses, all recombinants were scored after 88 h of incubation Recombination frequencies (*xyl*$^+$ or *metE*$^+$*metA*$^+$) are expressed per Hfr donor after subtracting unmated revertants Each number represents the mean of two to four independent experiments (the range was up to fourfold)

This conclusion follows from the evidence that the MutH protein recognizes and cleaves unmethylated GATC sequences in the course of methyl-directed mismatch repair[30,32] Note, however, that the requirement for unmethylated GATC sequences and MutH protein is alleviated when strand discontinuities flank the mismatch-containing region[30,32]

To assess whether *Escherichia mut*$^+$ genes replaced *Salmonella mut*$^-$ genes, we monitored the transposon (Tn) insertions in the *Salmonella mut* genes if the functional *E coli mut*$^+$ gene replaced the resident Tn-bearing *mut* gene, then the recombinant will have lost both its Tn-borne drug resistance and its mutator character This was so for seven out of eight stable *xyl*$^+$ *met*$^+$ recombinants from the cross with *S typhimurium mutL* Tn*10* as the recipient, indicating that except in one recombinant, *Escherichia* sequences had replaced *Salmonella* sequences for ~5 min beyond the last selected gene (*metA*) into the *mutL* region (see Fig 1) None of 27 unstable *xyl*$^+$ *met*$^+$ recombinants from the same cross lost drug resistance or the mutator phenotype (Table 3)

In the cross with *S typhimurium mutU* Tn*5* as the recipient, the *mutU* gene was replaced in all of the 19 stable *xyl*$^+$ recombinants (17 of which were also *met*$^+$ the *xyl* and *met* loci flank *mutU*, see Fig 1) Also, out of 16 unstable recombinants, 3 also showed *mutU* replacement, indicating that coincident replacement and duplication occurred None of the *xyl*$^+$ *met*$^+$ recombinants from *mutS* Tn*10* and *mutH* Tn*5* crosses showed transposon loss or *mut*-gene replacement, because mating was interrupted at 40 min and the *mutS*-*mutH* chromosomal region of genes was therefore unable to be transferred (see Fig 1) The stability of *xyl*$^+$ recombinants from *mut*$^+$ and *mutU* crosses does not prove that gene replacement occurs because heterospecific duplications could be stable owing to very rare homeologous recombination in *mut*$^+$ and *mutU* strains (Table 2)

Additional evidence for the physical linkage of *E coli* and *typhimurium* DNA sequences comes from the study of restrictio enzyme digests of parental and recombinant genomes DNA the parental strains and three stable recombinants (one ea from *mutH*, *mutL* and *mutS* crosses) was cut with *SpeI* enzym (Fig 2) The two largest fragments (together, ~1 megabas from parental *S typhimurium* recipient are lost in all thre recombinants and each of the recombinants has at least on nonparental fragment, which presumably contains an inte species junction Two of the recombinants have a large fragme which co-migrates with a large *E coli* fragment

Transduction

Transduction experiments were conducted by using phage P2 to move either *E coli* or *S typhimurium* histidine operon sequences between two *S typhimurium* strains The various recipient carried insertion sequences—IS*200* (ref 33) for the experiment of Table 4A and Tn*10-dCam* (ref 34) for those in Table 4B—in the *hisD* gene and also a deletion in an unlinked locu (*proA, B*) essential for proline biosynthesis The donor fo heterospecific transduction had a deletion of most of the *hi* locus[35], but was a histidine prototroph because it harboured 150-kilobase (kb) *his*$^+$ F′ episome from *E coli* Thus, the *hi* locus in the donor strain was uniquely encoded by *E co* sequences, whereas the *pro* locus was the normal *S typhimuriu* sequence Transducing lysate for homospecific crosses was prepared on wild-type *S typhimurium* and thus containe *S typhimurium* sequences at both the *his* and the *pro* locus comparison of the *hisD*$^+$ *proA*$^+$*B*$^+$ transduction ratios from th two lysates gives a measure of the efficiency of interspecie recombination relative to intraspecies recombination Th results (Table 4) are qualitatively similar to the results of conjugation experiments, in that *mutL* and *mutS* have the stronges effect and *mutU* the smallest effect Because the *E coli* sequence used in these transduction experiments were obtained from a episome present in *S typhimurium*, the effect of the mismatc repair system must be related only to sequence difference between the species rather than to a species-specific modificatio of the DNA

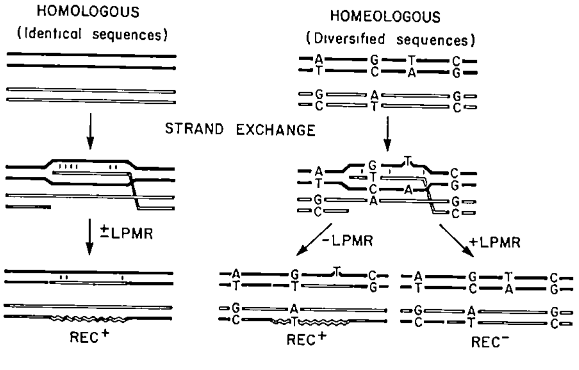

FIG 3 Molecular model for selective anti-recombination by LPMR (long-patch mismatch repair) proteins Homologous (identical) sequences recombine with the same efficiency in the presence or absence of active LPMR proteins (Table 4, and ref 9) Homeologous (diversified) sequences start to recombine by forming an heteroduplex containing mismatches caused by sequence differences in the two parental DNAs If LPMR proteins are not functional, homeologous recombination proceeds to form recombinant products (REC$^+$ phenotype) If LPMR proteins are functional mismatches in the heteroduplex regions are recognized, the reaction is reversed by the removal or destruction of the heteroduplex intermediate, and no viable recombinants arise (REC$^-$ phenotype) Although irrelevant to the mechanism of anti-recombination, the initiation of strand exchange is arbitrarily shown in a single-strand invasion mode[63] Only base-pair differences between the parental molecules are indicated the hybrid region in this sketch (shown with dashes indicating hydrogen bonding) contains one G T mismatch plus one unpaired T residue (as in a frameshift mutation)

Evidence for heterospecific gene replacement is shown in Table 4B The *hisD* allele in the recipient contains a Tn*10* derivative that confers chloramphenicol resistance Only 1 of 18 *hisD*$^+$ transductants retained chloramphenicol resistance All of 400 *proA*$^+$*B*$^+$ transductants retained chloramphenicol resistance A Mu*d-lacZ* fusion to *hisD* is likewise replaced during heterospecific recombination by P22 transduction (data not shown) Because the homospecific recombination by P22 ransduction occurs by integration of double-stranded DNA[36], he *mutH* effect in our experiments could have been due to repair synthesis generating new undermethylated DNA in *cis* o the incoming DNA

Discussion

The results reported here indicate that the suppression of mismatch repair can allow interspecies recombination between sequences that have as much as 20% sequence divergence Evidence for an inhibitory action of the *E coli* mismatch repair system on recombination between diverged sequences has been found in three other experimental systems First, by using a very short patch (vsp) mismatch repair system as a means to measure the formation of heteroduplex DNA intermediates *in vivo* it was found that the *mutH-mutU* system suppresses recombinant formation in bacteriophage λ crosses owing to the presence of two genetic markers[14] Second, carrying out crosses between a phage-λ vector and a plasmid, each bearing a mouse *V*H immunoglobin gene sequence, in a *mutS* host was found to increase recombination between partially homologous, but not between identical, *V*H genes[15] Finally, Karimova *et al* presented evidence indicating that recombination between two highly diverged lambdoid prophages can be enhanced in mutator hosts[10]

We favour proposals in which recombination between partially homologous sequences involves a reversible heteroduplex intermediate that is detected and aborted through a process that involves mismatch recognition[8,9] (see Fig 3) This is the stage at which genetic recombination is limited in transformation of *S pneumoniae* by the Hex mismatch repair system[16 17] The alternative proposal involves the mismatch-stimulated killing[11-13] of the products of recombination between diverged sequences[10 15] Although the mechanism by which mismatch repair proteins act to suppress recombination has not been elucidated, it seems that they edit not only the products of DNA replication[20-22], but also intermediates in the process of genetic recombination

There is evidence that mismatch repair also operates in fungal[37], amphibian[38] and mammalian[39] cells The average sequence divergence among individual copies of the major dispersed repetitive sequence families in mammalian genomes, such as the *Alu* sequences[40], is similar to the sequence divergence between the genomes of *E coli* and *S typhimurium* The mismatch repair system could act to prevent inter- and intrachromosomal rearrangements by suppressing recombination between misaligned dispersed repetitive sequences and thus play an important part in maintaining gene order in bacteria[41] and in eukaryotes Similarly, if the recombination suppressing effect of diverged sequences extends into neighbouring regions of perfect homology, then the high sequence polymorphism among homologous chromosomes in humans[42] could contribute to the suppression of mitotic recombination without compromising the ability of the cells to use recombinational DNA repair involving exchanges between sister chromatids of identical sequences (sister chromatid exchange) Mitotic recombination leads to the expression of deleterious recessive mutations which are often associated with pathological states such as cancer[43]

Some regulation of the mismatch repair system, or of the extent of heteroduplex DNA formation, would presumably be necessary during meiosis to allow pairing and recombination between homologues in spite of their sequence polymorphism If meiosis becomes deficient in chromosome pairing, for example, when sequence divergence is sufficiently extensive and a mismatch repair system is sufficiently active, chromosome nondisjunction will result in sterility[44] Fertility can then be restored through inbreeding or selfing, thus avoiding sequence divergence, or by transiently inactivating the mismatch repair system[45,46] The result would be the emergence of a new species Thus, mismatch repair could be a molecular mechanism for maintaining a genetic barrier between species that have diverged, but not drastically, on the DNA sequence level, and could accomplish the genetic separation (that is, reproductive isolation) required for evolution of species in the absence of geographical isolation[47] The acquisition of dispersed noncoding DNA, such as repetitive sequences and introns, could accelerate speciation by allowing an increased rate of genomic sequence divergence and subsequent meiotic and mitotic recombinational isolation of genes, chromosomes and species An alternative chromosomal mechanism for speciation is the accumulation of large chromosomal rearrangements An extensive study of a marsh rat population showed that chromosomal rearrangements, however, have a smaller role in reproductive isolation than that previously believed[48]

Mismatch repair apparently helps to prevent all classes of genetic alterations, it prevents point mutations by correcting replication errors[16 20-22] and chromosomal rearrangements by preventing recombination between sequences of only limited homology (limited in size[41] or extent—this work and ref 15) Modulation of the mismatch repair system[45 46] provides an effective way to control the rate of 'vertical' evolution (occurring by means of new point mutations and intrachromosomal rearrangements) and 'horizontal' evolution (occurring through recombination with diverged sequences)

This work shows that suppression of mismatch repair allows interspecies recombination between bacteria that have been diverging for ~150 million years[19] Hybrids were formed between *E coli* and *S typhimurium* that are in fact new species with only partial fertility in crosses with each of two parental

TABLE 3 Analysis of *xyl*$^+$ and *met*$^+$ recombinants from *E coli* Hfr × *S typhimurium* F$^-$ conjugational crosses

F$^-$ recipient	Co-inheritance of markers % *met*$^+$ among *xyl*$^+$	% *xyl*$^+$ among *met*$^+$	*xyl*$^+$ recombinants Stable unstable	Transposon loss
S typhimurium				
mut$^+$	7 7 (389)	5 8 (412)	33 1	
mutL Tn*10*	3 6 (412)	9 4 (412)	8 27	7/8, 0/27
mutS Tn*10*	8 7 (412)	6 6 (375)	1 34	0/1, 0/34
mutH Tn*5*	10 7 (412)	1 9 (412)	7 28	0/7, 0/28
mutU Tn*5*	2 5 (412)	22 0 (412)	19 16	19/19, 3/16
E coli				
mut$^+$	63 1 (412)*	18 9 (412)*	20 0	

To determine co-inheritance of markers, *xyl*$^+$ or *met*$^+$ recombinants were replicated on media selecting for *met*$^+$ or *xyl*$^+$ bacteria, respectively, and scored after 2 days of incubation Total numbers of colonies analysed are given in parentheses The stability of *xyl*$^+$ recombinants was tested by growing each colony in a rich liquid medium and streaking on 1% xylose McConkey agar, on which *xyl*$^+$ bacteria give red colonies and *xyl*$^-$ bacteria white colonies For each recombinant, a red colony was grown and streaked a second time The recombinants that then gave only red colonies were scored as stable, whereas those giving some white, dark red or sectored colonies were scored as unstable Numbers are of recombinants scored Transposon loss was tested by replicating each of the recombinants previously scored for stability onto a rich medium containing tetracycline (12 5 μg ml^{-1}) for Tn*10* or kanamycin (50 μg ml^{-1}) for Tn*5*, and scoring resistant colonies after one day of incubation The first and second fractions represent the number of stable and unstable recombinants, respectively, expressing drug sensitivity All recombinants that lost the original Tn*10* or Tn*5* were shown in subsequent tests to have also lost the mutator phenotype (data not shown)

* An *arg*$^+$ marker used instead of *met*$^+$, the two are closely linked

TABLE 4 Transduction crosses

A Mutator alleles enhance the ability of *E coli* sequences to donate histidinol prototrophy to *S typhimurium*

Recipient strain *mut* allele	Heterospecific crosses *hisD*$^+$ *E coli* *proA*$^+$*B*$^+$ *S typhimurium* *hisD*$^+$/*proA*$^+$*B*$^+$ (×10^4)	Homospecific crosses *hisD*$^+$ *S typhimurium* *proA*$^+$*B*$^+$ *S typhimurium* *hisD*$^+$/*proA*$^+$*B*$^+$
mut$^+$	4	320
mutL no 1	355	180
mutL no 2	147	150
mutS no 1	696	130
mutS no 2	451	160
mutU no 1	20	460
mutU no 2	10	520
mutH no 1	37	180
mutH no 2	170	190

B Interspecific transduction by allelic replacement

Recipient strain *mut* allele	Plate contains	Plate selects for	Mean no of colonies
mut$^+$	Pro, Hol	His$^+$	5
	Pro, Hol, Cam	His$^+$, Cam^r	0
	His, Arg	Pro$^+$	478
	His, Arg, Cam	Pro$^+$, Cam^r	498
mutS	Pro, Hol	His$^+$	168
	Pro, Hol, Cam	His$^+$, Cam^r	0
	His, Arg	Pro$^+$	1,344
	His, Arg, Cam	Pro$^+$, Cam^r	1,110

In part A, the recipient for P22 transduction was *hisD984* IS*200 proAB47* where *hisD* IS*200* is a nonreverting transposable element insertion[33] and *proAB47* is a nonreverting deletion of genes essential for proline prototrophy[59] The donor P22 Hft *int3* lysate[60] carrying *E coli hisD* sequences and *S typhimurium proAB* sequences was grown on strain TR5360 from John Roth's strain collection, although strain TR5360 contains *his*646, a deletion covering the *hisD* gene[35], it is a histidinol prototroph because it also carries *E coli* F′ 150 which includes the entire *E coli his* operon[61] All *S typhimurium* strains are in the LT2 background, although the *proAB*47 allele was first isolated in LT7 and subsequently moved into LT2 The P22 donor lysate with *S typhimurium* sequences at both the *his* and the *pro* loci was prepared on LT2 No 1 and 2 refer to two independent *mut*(*H, L, S* or *U*) derivatives of the initial recipient strain, these were obtained by P22-mediated transduction (as in Table 1) of mutator alleles from SL4213 *mut* strains (Table 1) Selection was conducted by spreading 0 1 ml of donor lysate and 0 1 ml of a fresh overnight culture of recipient cells on a minimal-glucose plate, the plates were scored after 2–3 days at 37 °C Aliquots of the same overnight culture and P22 donor lysate were used for both the histidinol (His$^+$) and the proline (Pro$^+$) selections When His$^+$ was selected, the plates contained proline and histidinol, for Pro$^+$ selection, the plates contained both histidine and arginine, the arginine prevents *argD* mutants from acting as indirect suppressors of proline auxotrophy[62] In part B, the recipient was *proAB47 hisD* Tn*10d-Cam* (which confers chloramphenicol resistance as well as histidinol auxotrophy[34]) *E coli* sequences donated *hisD*$^+$ and *S typhimurium* sequences donated *proAB*$^+$ Transductions were conducted as for part A Undiluted donor lysate (0 1 ml) was used for transductions to histidinol prototrophy and 0 1 ml of a 10-fold dilution of the lysate was used for transductions to proline prototrophy The numbers in the last column are the means of independent experiments, ranges were less than twofold Precise and nearly precise excision of transposons are increased in mutator backgrounds[41] but the insertion alleles used here are not susceptible to mutator-enhanced excision generating prototrophs neither the *hisD984* IS*200* nor the Tn*10d-Cam* allele reverted, even in mutator backgrounds (data not shown) Cam, chloramphenicol, Cam^r, chloramphenicol-resistant phenotype, Hol, histidinol

organisms The utility of technologies allowing interspecies recombination is wide ranging For example, attenuating the pathogenicity of *Salmonella, Shigella* or other pathogenic species by substituting appropriate genomic sequences from related nonpathogens (such as *E coli*) could allow the production o hybrid species suitable for live vaccines Interspecies transfe of interesting alleles should facilitate genetic analysis of relate species Easy selection for gene replacements, duplications an amplifications after conjugation could provide useful micro-organisms in which the duplicated heterospecific region is stabilized by converting recombinants to *mut*′ (Table 3) In addition interspecies genetics could facilitate studies on the structure and function of hybrid operons (Table 4), genes and proteins[5] and the production of proteins with altered activity, stability or specificity For example, recombination between diverged genes in *mutL, S* bacteria should create complex heteroduplex regions which are substrates for diverse specialized vsp mismatch repair systems (see ref 9 for a review) generating complex patchworks of the two parental sequences[49] By recombining and patchworking functionally equivalent but sequence-diverged genes cloned from different species, it should be possible to create new diversified genes that still conserve their basic motif and yet alter stability or specificity of the gene products This strategy for protein 'engineering' could be similar to hyperconversion mechanisms generating diversity in the expressed genes by their recombination with diverged silent (pseudo)genes (see ref 50 for a review), as in the case of immunoglobin genes[51] and genes coding for variable surface proteins in parasites[52,53] □

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1 Holliday R *Genet Res* **5**, 282–304 (1964)
2 Whitehouse H L K *Nature* **199**, 1034–1040 (1963)
3 Fox M S & Allen M *Proc natn Acad Sci USA* **52**, 412–419 (1964)
4 Meselson M in *Heritage from Mendel* (ed Brink R A) 3–16 (University of Wisconsin Press 1965)
5 Schneider W P Nichols B P & Yanofsky C *Proc natn Acad Sci USA* **78**, 2169–2173 (1981)
6 Shen P & Huang H V *Genetics* **112**, 441–457 (1986)
7 Das Gupta C & Radding C M *Nature* **295**, 71–73 (1982)
8 Radman M in *Genetic Recombination* (eds Kutcherlapati R & Smith G R) 169–192 (American Society for Microbiology Washington DC 1988)
9 Radman M *Genome* **31**, 68–73 (1989)
10 Karimova G A Grigoriev P S & Rybchin V N *Molekularnaia Genetika Mikrobiologia i Virusologia* **10**, 29–34 (1985)
11 Glickman B W & Radman M *Proc natn Acad Sci USA* **77**, 1063–1067 (1980)
12 Wagner R *et al Cold Spring Harb Symp quant Biol* **49**, 611–615 (1984)
13 Doutriaux M P Wagner R & Radman M *Proc natn Acad Sci USA* **83**, 2576 2578 (1986)
14 Jones M Wagner R & Radman M *Cell* **50**, 621–626 (1987)
15 Shen P & Huang H V *Molec Gen Genet* **218**, 358–360 (1989)
16 Claverys J P & Lacks S A *Microbiol Rev* **50**, 133–165 (1986)
17 Mejean V & Claverys J P *Molec Gen Genet.* **197**, 467–471 (1984)
18 Feinstein S I & Low K B *Genetics* **113**, 13–33 (1986)
19 Ochman H & Wilson A C in Escherichia coli *and* Salmonella typhimurium (ed Neidhardt F C) 1649–1654 (American Society for Microbiology Washington DC 1987)
20 Radman M & Wagner R *A Rev Genet* **20**, 523–538 (1986)
21 Modrich P *A Rev Biochem* **56**, 435–466 (1987)
22 Meselson M in *The Recombination of Genetic Material* (ed Low B K) 91–113 (Academic New York 1988)
23 Kushner S R *et al UCLA Symp molec cell Biol* **11**, 153–159 (1983)
24 Hickson I D *et al Molec Gen Genet* **190**, 265–270 (1983)
25 Kumura K & Sekiguchi M *J biol Chem* **259**, 1560–1565 (1984)
26 Baron L S *et al Bacteriol Rev* **32**, 362–369 (1968)
27 Middleton R B & Mojica a T *Adv Genet* **16**, 53–79 (1971)
28 Anderson P R & Roth J R *A Rev Microbiol* **31**, 473–505 (1977)
29 Lehner A F & Hill C W *Genetics* **110**, 365–380 (1985)
30 Lahue R S Au K G & Modrich P *Science* **245**, 160–164 (1989)
31 Siddiqi O & Fox M S *J molec Biol* **77**, 101–123 (1973)
32 Laengle Rouault F Maenhaut-Michel G & Radman M *EMBO J* **6**, 1121–1127 (1987)
33 Lam S & Roth J R *Cell* **34**, 951–960 (1983)
34 Eliott T & Roth J R *Molec Gen Genet* **213**, 332–338 (1988)
35 Hartman P E Hartman Z & Stahl R C *Adv Genet* **16**, 1–34 (1971)
36 Ebel-Tsipis J Fox M S & Botstein D *J molec. Biol* **71**, 449–469 (1972)
37 Bishop D K Andersen J & Kolodner R D *Proc natn Acad Sci USA* **86**, 3713–3717 (1989)
38 Brooks P *et al Proc natn Acad Sci USA* **86**, 4425–4429 (1989)
39 Brown T C & Jiricny J *Cell* **54**, 705–711 (1988)
40 Britten R J *et al Proc natn Acad Sci USA* **85**, 4770–4774 (1988)
41 Lundblad V & Kleckner N *Genetics* **109**, 3–19 (1985)
42 White R & Lalouel J M *Sci Am* **258**, 40–48 (1988)
43 Weinberg R A *Cancer Res* **49**, 3713–3721 (1989)
44 Murray A W & Szostak J W *A Rev cell Biol* **1**, 289–315 (1985)
45 Damagnez V , Doutriaux M P & Radman M *J Bact* **171**, 4494–4497 (1989)
46 Schaaper RM & Radman M *EMBO J* **8**, 3511–3516 (1989)
47 Barton N H Jones J S & Mallet J *Nature* **336**, 13–14 (1988)
48 Nachman M W & Myers P *Proc natn Acad Sci USA* **86**, 6666–6670 (1989)
49 Abastado J P *et al Proc natn Acad Sci USA* **81**, 5792–5796 (1984)
50 Maizels N *Trends Genet* **5**, 4–8 (1989)
51 Radman M in *Cellular Responses to DNA Damage* (eds Friedberg E C & Bridges B A) 287–298 (Liss New York 1983)
52 Thon G Baltz T & Eisen H *Genes Dev* **3**, 1247–1254 (1989)
53 Seifert H S *et al Nature* **336**, 392–395 (1988)
54 Bachmann B J in Escherichia coli *and* Salmonella typhimurium (ed Neidhardt F C) 807–844 (American Society for Microbiology Washington DC 1987)

55 Kahn P L *J Bact* **96,** 205-214 (1968)
56 Sanderson K E & Stocker B A D in Escherichia coli *and* Salmonella typhimurium (ed Neidhardt F C) 1220-1224 (American Society for Microbiology Washington DC 1987)
57 Shanabruch W G Belau I & Walker G C *J Bacteriol* **147,** 827-835 (1981)
58 Miller J H *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory New York 1972)
59 Csonka L N *Molec Gen Genet* **182,** 82-86 (1981)
60 Schmieger H *Molec Gen Genet* **119,** 75-88 (1972)
61 *Low K B Bact Rev* **36,** 587-607 (1973)
62 Leisinger T in Escherichia coli *and* Salmonella typhimurium (ed Neidhardt F C) 345-351 (American Society for Microbiology Washington DC 1987)
63 Meselson M & Radding C M *Proc natn Acad Sci USA* **72,** 358-361 (1975)

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Extragalactic radiation and the ultra-high-energy cosmic-ray spectrum

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The high-frequency (submillimetre) side of the 'black-body' radiation spectrum has now become available for study from balloons and the soon-to-be-launched Cosmic Background Explorer (COBE) satellite There is recent evidence of an unexpectedly large excess of radiation beyond the peak of the black-body curve[1], which seems to be generally isotropic[2] and therefore of extragalactic extent. Such radiation will have a significant effect on the propagation of ultra-high-energy cosmic rays. Because of the importance of this new discovery and the timeliness of the subject, I re-examine here the effect of extragalactic microwave and submillimetre-radiation fields on the ultra-high-energy cosmic-ray spectrum. Although the most detailed work[3,4] so far is based on complex numerical simulations of individual interactions, I find that the general characteristics of the spectrum can be derived from fairly simple analytical arguments I show that one can explore, by simpler and more general means, the various spectral features obtained by numerical calculations ('pile-up' bumps and 'dips'[3,4]). I illustrate this technique with a newly derived lifetime–energy relation, based on the new submillimetre observations.

Semi-analytical methods can be useful in examining the implications of forthcoming data on background radiation fields without computer simulations involving specific parameterizations Such features can be obtained directly from a consideration of the average lifetime curves[5-7] and the phase-space continuity equation For simplicity, I consider that the highest-energy cosmic rays are protons (Similar considerations can be extended to nuclei, but that case is complicated by a time-variable charge-dependent pair-production loss rate owing to the photodisintegration process[3]) The effective lifetime of such cosmic rays against energy loss by photomeson production (or equivalent mean-free-path) on black-body radiation of temperature, T_{B}, is given by[5]

$$\tau^{-1}(E)=(1/4\pi)\gamma^2h^3c^2\int_{\varepsilon_{\mathrm{th}}/2\gamma}^{\infty}\mathrm{d}\varepsilon(\exp(\varepsilon/kT_{\mathrm{B}})-1)$$

$$\times\int_{\varepsilon'_{\mathrm{th}}}^{2\gamma\varepsilon}\mathrm{d}\varepsilon'\,\sigma_{\gamma\mathrm{p}}(\varepsilon')K(\varepsilon') \qquad (1)$$

where γ is the proton Lorentz factor, ε' is the photon energy in the rest frame of the ultra-high-energy proton and the inelasticity, $K(\varepsilon')$, is the mean energy lost by photomeson production per interaction

Equation (1) for a pure black-body spectrum can easily be extended to include the recently discovered submillimetre-radiation field by using the parameterization of a pure black-body field and a further submillimetre gray-body radiation field[8] of temperature $T_{\mathrm{G}} = 4\ 9$ K and dilution factor 0 04 The cross-section data originally used in ref 5 have been updated using recent compilations of the Particle Data Group[9] Pair-production losses[5] have also been included to obtain the total lifetime curve Figure 1 shows the function $\tau(E)/E$, which is the inverse energy loss rate in GeV^{-1} s The solid curve includes the effect of the submillimetre radiation and the dashed curve indicates the effect of the black-body radiation field only

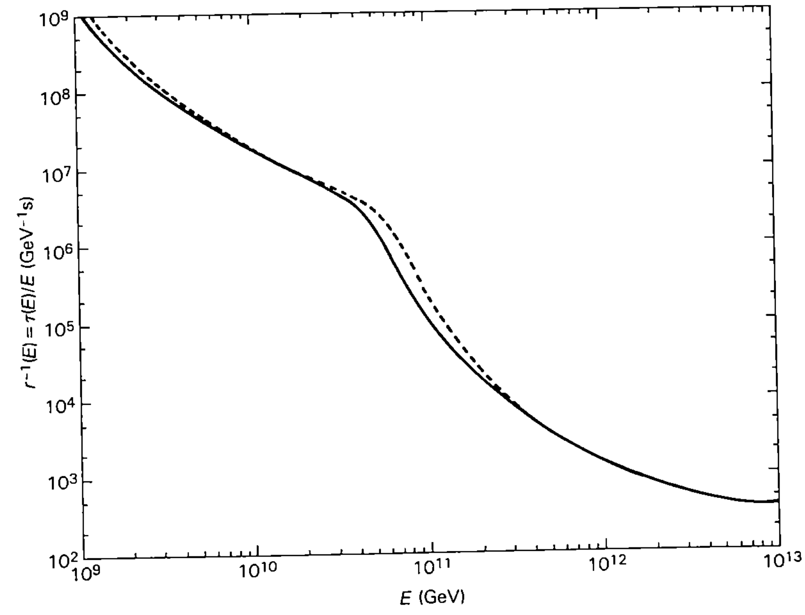


FIG 1 The inverse energy loss rate $r^{-1}(E)$ produced by the black-body radiation field only (dashed line) and the black-body radiation field plus the submillimetre radiation field (solid line)

I note that the mean lifetime curve itself (which can be obtained from Fig 1) is the source of much information First, it indicates that if the sources of ultra-high-energy cosmic rays are of cosmological origin, from quasars or primordial galaxies for example, protons of energies larger than $\sim 10^{10}$ GeV will be degraded by energy losses from pair production and photomeson production[10 11] Second, protons in the energy range $\gtrsim 10^{11}$ GeV should have a 'local' origin, most likely from sources within the local Virgo Supercluster, if the spectrum shows no attenuation effects at the highest energies[5] The local-supercluster-origin model first suggested in ref 5 has been developed elsewhere[12,13]

Figure 1 can be used as a starting point for a further examination of the spectral features to be expected, given an ultra-high-energy cosmic-ray source function $q(E, t)$ The quantity $\tau(E)$ defined in equation (1) should not be misinterpreted It represents a characteristic energy loss time for the protons, not a decay time for catastrophic loss In fact, because of the inclusion of the integration over the inelasticity term $K(\varepsilon')$, $\tau(E)$ can be rewritten so that a simple formalism that automatically conserves proton number can be used To do this, I first define a function describing the mean energy loss rate from pair production and photomeson production interactions, $r(E) \equiv \mathrm{d}E/\mathrm{d}t$ so that

$$r(E) = E/\tau(E) \qquad (2)$$

and the quantity τ/E plotted in Fig 1 is actually the inverse of the mean proton energy loss rate Conservation of proton number is then assured by the relation

$$\frac{\partial n(E, t)}{\partial t} + \frac{\partial}{\partial E}[n(E, t)r(E)] = q(E, t) \qquad (3)$$

which describes a divergenceless flow of cosmic-ray protons in energy space in the case for which I assume spatial isotropy and no catastrophic losses In this case, the cosmic-ray flux is directly proportional to the proton number density

$$I(E, t) = n(E, t)c/4\pi \qquad (4)$$

Equations (3) and (4) have the solution[14]

$$I(E, t) = \frac{\tau(E)c}{4\pi E}\int_E^\infty \mathrm{d}E'\, q\left(E', t - \int_E^{E'} \frac{dE''}{E''}\tau(E'')\right) \qquad (5)$$

In equation (5), the quantity $\tau(E)/E$ has been substituted for the inverse of the absolute value of the energy loss rate, as follows from equation (2) Equation (5), which was obtained using Green's function techniques, clearly indicates that the cosmic-ray spectrum at time t is obtained from two factors These are a term that is the inverse of the energy loss rate at energy E, which accounts for the 'trickle out' to energies below E and is the factor given in Fig 1, and a 'trickle down' input factor given by the integral over source spactra from earlier times

$$t' = t - \int_E^{E'} \frac{\mathrm{d}E''}{E''}\tau(E'') \qquad (6$$

and higher energies $E' > E$, for which the initial energies have been degraded by energy losses For example, it follows from the the results in Fig 1 that it takes $\sim 10^9$ yr for a $\sim 10^{11}$ GeV proton to be degraded to an energy of $\sim 4 \times 10^{10}$ GeV It is the effect of the 'trickle down' integral that can cause a 'pile-up' in the spectrum

The treatment I use here breaks down at energies $\lesssim 10^9$ GeV, the point at which the energy loss time becomes comparable to or greater than the Hubble time For cosmic rays of truly cosmological origin, the age of which is a significant fraction of the age of the Universe, energy losses and dilution effects from the expansion of the Universe have a critical role In this case, the first term in equation (3) must be replaced by a derivative with respect to redshift[7,14] I will not pursue the subject of the cosmological cosmic rays here as it is beyond the scope of this paper and does not seem to bear on the origin of the highest-energy cosmic rays[5]

As illustrations of how this formalism can be used, I consider a steady-state solution of equation (3), and a 'burst' delta-function source spectrum that has been 'aged' by a specific amount (refs 3, 4)

To find the steady-state time-independent solution, I set the $\partial n/\partial t$ term in equation (3) equal to zero Equation (5) then becomes

$$I(E) = \frac{\tau(E)}{E}\int_E^\infty \mathrm{d}E'\, q(E') \qquad (7)$$

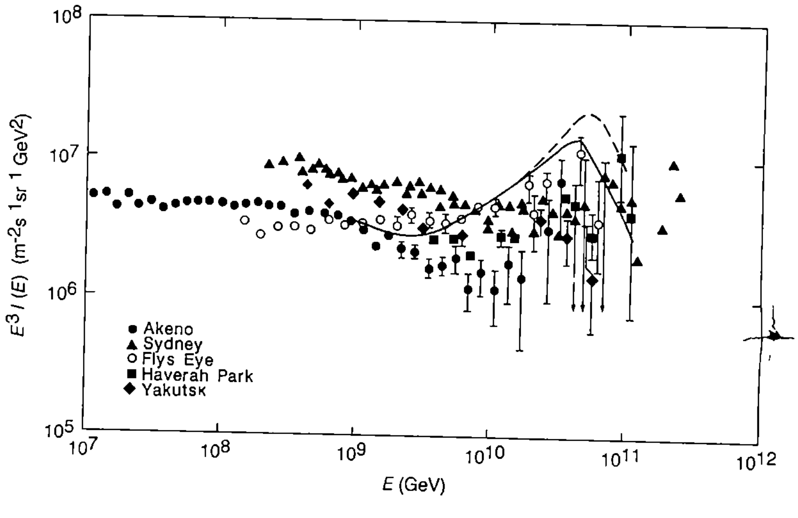
which can be integrated if $q(E)$ is a power-law spectrum $KE^{-\Gamma}$ In this case

$$I(E) = \frac{q(E)\tau(E)}{\Gamma - 1} \qquad (8)$$

Thus, the factor $\tau(E)$ has the role of a modulation factor on the initial source spectrum It is as if the cosmic rays of energy E were built up over a time given by $\tau(E)/(\Gamma - 1)$ The flux at energy E becomes proportional to the energy loss time at energy E as well as the source flux at energy E

Figure 2 shows the form of the spectrum obtained by an application of equation (8) in the case of an E^{-2} source spectrum such as might be expected if the highest-energy cosmic rays are produced by shock acceleration in extragalactic sources[15] The spectrum is superimposed on a compilation of recent air-shower data[16-20] A 'pile-up' effect between 2 and 5×10^{10} GeV can

FIG 2 The cosmic-ray flux spectrum derived from various air-shower results shown with the shape of the steady-state equilibrium spectrum obtained from equation (8) (Solid and dashed lines as in Fig 1)



seeing was ~1 5 arcsec in diameter Although the broad component, which is due to the supernova itself, originated in an unresolved point source, approximately half of the total intensity of the narrow component arose in a compact source or 'knot' displayed by 0 8 ± 0 3 arcsec at position angle 200 ± 15° The knot diameter was ≤1 5 arcsec (1 2 light yr) A faint northerly extension to the emission was also seen We also made north–south line scans through supernova 1987A, using the same spectral and spatial sampling, and a similar displacement in declination was seen (Fig 3) Clearly, most of the emission in the narrow component had a circumstellar origin

Several observations indicate that the compact source lay behind the supernova, and so had been observable for less than the supernova lifetime First, circumstellar gas at the source position angle is receding[8] Second, contemporary mid-infrared measurements (P F Roche, D K Aitken and C H Smith, personal communication) revealed supernova-illuminated dust lying in a compact region displaced from the supernova and, to within the errors, coincident with the He I knot Because dust cools virtually instantaneously, its emission maps out the present location of energy from the light-curve peak Considerations of geometry, light travel time and temperature indicate that the dust must lie behind the supernova[20,21] Third, direct optical continuum imaging around day 750 indicates scattering from dust with a region of enhanced surface brightness at about the same sky position as the He I knot[17] Geometry and light-travel-time effects again point to a location behind the supernova Optical speckle observations[22] in a continuum bandpass also show a source at about the same position from days 644 to 697, although there seems to be some disagreement between direct and speckle imaging as to the position and magnitude of the brightest region[17,23 24] Fourth, the high density derived below shows that the source did not comprise the material emitting the ultraviolet lines during the first 1 1 years

We have constructed a simple model of the compact He I source consisting of a slowly moving cloud of hydrogen and helium only We assume that it was fully ionized by the ultraviolet flash, and that light travel time across it was unimportant Our model predicts almost constant He I 1,083-nm emission per unit volume from 3 months to 1 year after initial ionization From the constancy of the observed intensity we conclude tha the mass of emitting gas did not increase significantly durin the period of observation, so that by day 737, we had bee viewing the same cloud for at least 0 8 yr If we adopt a viewi time of 1 yr since ionization, the cloud lies 0 8 light yr from t supernova

We have allowed for recombination, collisional effects an ionization of neutral atoms by photons released in the recombination of He^{2+} We take the He I line to be optically thin an use atomic parameters from refs 25–30 An electron temperatur of ~25,000 K was inferred from approximately contemporar intensity ratios of the narrow 436 3, 495 9 and 500 7-nm [O III lines (R Stathakis, personal communication) A higher temperature was found initially[34], but cooling would have bee rapid[11] Only some of the [O III] emission had the same sk position as the knot[17], and so we do not know if the [O III] derived temperature is appropriate for the helium cloud W therefore adopted 30,000 K as a conservative upper limit for th cloud temperature—a lower temperature would increase th inferred helium overabundance Our only free parameters wer the electron density N_e and the helium mass fraction Y W corrected for interstellar reddening[32,33] and included contributions of He II to the Balmer lines

We have compared the model with our He I observations an with the intensities of narrow Hα, Hβ and He II 469-nm line obtained from A P S Crotts (personal communication) an from the Anglo-Australian Observatory supernova archive (R Stathakis, personal communication) Around day 750, the tota narrow Hα intensity from the region within 3 arcsec of t supernova was 9×10^{-13} erg cm^{-2} s^{-1} The total narrow H intensity was 0 3 of this The He II 469-nm intensity decrease by about a factor of four between days 613 and 756 and ha approximately one-third of the total Hβ intensity on day 756 These intensities are un-dereddened

From images obtained around day 750, it is estimated (A P S Crotts, personal communication) that 0 3 ± 0 1 of the narrow Hα flux came from within the 1 5-arcsec maximum extent of the He I knot In comparing the model and observations, we therefore made the conservative assumption that 0 3 of the hydrogen emission originated from the cloud For $T = 30,000$ K,

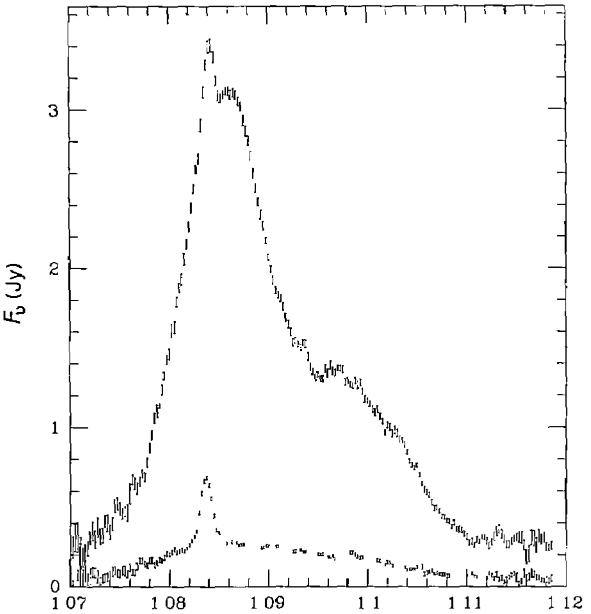


FIG 1 Spectra of supernova 1987A taken in September 1988 and January 1989 The broad component is helium and hydrogen emission from the supernova ejecta The narrow component is helium in the circumstellar shell

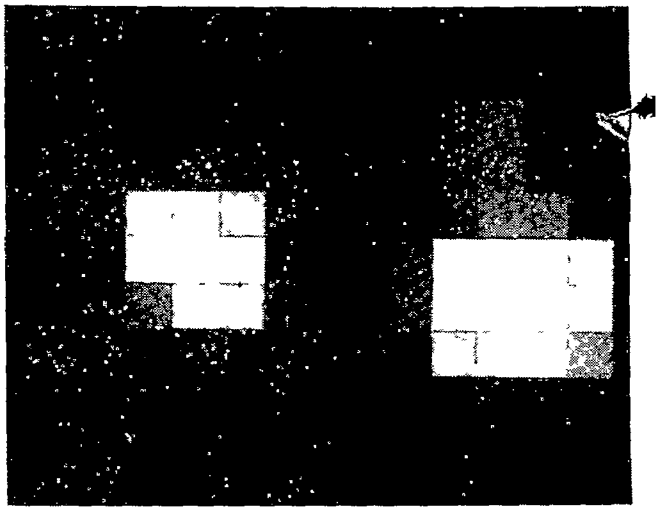


FIG 2 Grey-scale representations of maps of the broad (left) and narrow (right) components seen in Fig 1 North is at the top and east to the left The focal plane was sampled on a square grid, with 0 7-arcsec pixels, through a 1 4-arcsec square aperture The contribution of the broad component was removed from the observed flux in the narrow component by scaling from a fully sampled spectrum The resulting image shows the distribution of the narrow component alone Both maps have been automatically scaled to cover the full tonal range

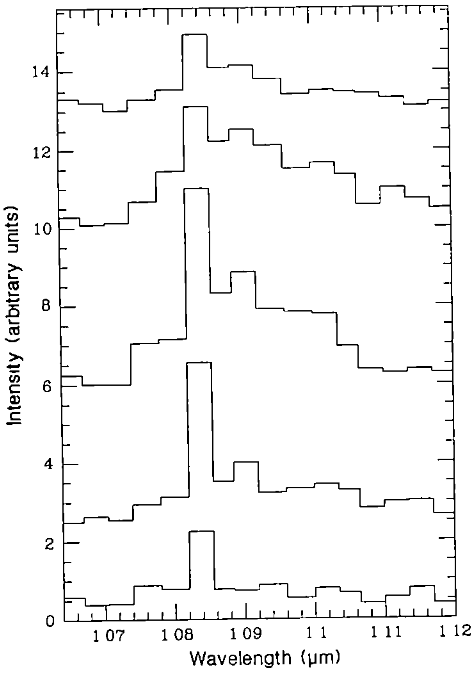


FIG 3 Six undersampled spectra from ~200 co-added declination scans through supernova 1987A, taken at 0 7-arcsec spacing The narrow component peaks in the second and third spectra from the bottom (south), and the broad component in the third and fourth

the model satisfactorily reproduces the observed fluxes if $N_e = 10^5$ cm^{-3} and Y is in the range 0 4-0 5 The corresponding N(H) is also $\sim 10^5$ cm^{-3} The 1,083-nm He I line arises both directly by recombination of ionized helium and from collisional excitation of the metastable 2^3S level. At $N_e \sim 10^5$, the latter dominates and the line is strengthened relative to the recombination case by more than an order of magnitude[25] The cloud mass density is about 10 times larger than the typical value for the whole shell obtained from ultraviolet observations[6] From the He I line intensity we derive a total mass of $\sim$0 01 $M_\odot$ in the cloud This is a factor of 5-10 less than the shell mass inferred from the ultraviolet lines[6,11]

In the plane of the sky the shell extended to ~2 light yr from the supernova[16,17,31] and had lower density[6] The cloud could not have been produced by the second (blue supergiant) wind, for if it had expanded into an isotropic shell it could only have accumulated high densities at larger radii The compact He I source may be an interstellar cloud that successfuly resisted the momentum flux of the red-supergiant wind However, it is unlikely that an interstellar cloud with so high a helium abundance would lie within 1 light yr of the supernova An alternative is a bipolar red supergiant wind[16], possibly also interacting with the interstellar medium A symmetrical bipolar form is incompatible with our data because the frontal lobe would now be seen at age 2 yr, yielding a 1,083-nm intensity 60% that of the rear lobe Moreover, any such frontal lobe must have been less dense than the rear if the density is to be compatible with the ultraviolet line ratios We therefore interpret the cloud as part of an asymmetric red-supergiant outflow An interacting outflow behind the supernova may account for the extended mid-infrared and continuum optical emission[17,20] Crotts *et al*[17] suggest that the circumstellar shell is near a dense flat sheet, possibly of interstellar origin, lying behind the supernova and point out that the He I knot lies close to where the shell and sheet intersect

Extensive mass loss and mixing of the progenitor envelope have been invoked to account for its blue-red-blue evolution before explosion and to explain the high N/C and N/O ratios inferred from the ultraviolet data[1-3] With the further constraint that the progenitor envelope was massive, Saio *et al*[2] find that complete mixing of the hydrogen-rich envelope together with dredge-up of about one solar mass of material from the helium core is required, giving a surface helium abundance ~0 45 Our observations support this model

Finally we note that the cloud subtends up to 2 sr at the supernova so the total number of photons capable of doubly ionizing helium and radiated isotropically in the ultraviolet flash must have been at least 10^{55}, in agreement with model predictions and ultraviolet observations[9-11] ☐

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1 Maeder A *ESO Workshop on the Supernova 1987A, Conf Workshop Proc* **26**, 251-269 (1987)
2 Saio H Nomoto K & Kato M *Nature* **334**, 508-510 (1988)
3 Woosley S E Pinto P A & Weaver T A *Proc astr Soc Aust.* **7**, 355-370 (1988)
4 Wamsteker W Gilmozzi R & Cassatella A *Int. astr Un Circ* **4410** (1987)
5 Wampler J *Int astr Un Circ* **4541** (1988)
6 Fransson C *et al Astrophys J* **336**, 429-441 (1989)
7 Chevalier R A *Nature* **332**, 514-516 (1988)
8 Chevalier R A *ESO Workshop on the Supernova 1987A Conf Workshop Proc* **26**, 481-494 (1987)
9 Dopita M A Meapheringham S J Nulsen P & Wood P R *Astrophys J* **322**, L85-L89 (1987)
10 Lundqvist P & Fransson C *ESO Workshop on the Supernova 1987A Conf Workshop Proc* **26**, 495-502 (1987)
11 Fransson C & Lundqvist P *Astrophys J* **341**, L59-L62 (1989)
12 Nisensen P Papaliolios C Karovska M & Noyes R *Astrophys J* **320**, L15-L18 (1987)
13 Meikle W P S Matcher S J & Morgan B L *Nature* **329**, 608-611 (1987)
14 Cropper M S *et al Mon Not R astr Soc* **231**, 695-722 (1988)
15 Papaliolios C *et al Nature* **338**, 565-566 (1989)
16 Wood P R & Faulkner D J *Int astr Un Circ* **4739** (1989)
17 Crotts A P S Kunkel W E & McCarthy P J *Astrophys J* (in the press)
18 Meikle W P S Allen D A Spyromilio J & Varani G F *Mon Not R astr Soc* **238**, 193-223 (1989)
19 Allen D Meikle P & Spyromilio J *Int. astr Un Circ* **4747** (1989)
20 Roche P F Aitken D K Smith C H & James D D *Nature* **337**, 533-535 (1989)
21 Felten J E & Dwek E *Nature* **339**, 123-125 (1989)
22 Karovska M Nisenson P Papaliolios C Standley C & Heathcote S *Int astr Un Circ* **4749** & **4752** (1989)
23 Crotts A & Kunkel W E *Int. astr Un Circ* **4753** (1989)
24 Heathcote S Suntzeff N & Walker A *Int. astr Un Circ* **4753** (1989)
25 Osterbrock D E *Astrophysics of gaseous nebulae and active galactic nuclei* (University Science Books Mill Valley 1989)
26 Jacobs V L & Burke P G *J Phys* B**5** 2272-2281 (1972)
27 Bell K L Kingston A E & Taylor I R *J Phys* **B6** 2271-2279 (1973)
28 Hata J & Grant I P *J Phys* B**14** 2111-2124 (1981)
29 Clegg R E S *Mon Not. R. astr Soc* **229**, 31P-39P (1987)
30 Clegg R E S & Harrington J P *Mon Not R. astr Soc* **239**, 869-883 (1989)
31 Wampler E J & Richichi A *The Messenger* **52**, 14-16 (1988)
32 Dopita M A *Proc astr Soc Aust* **7**, 344-351 (1988)
33 West R M Lauberts A Jørgensen H E & Schuster H-E *Astr Astrophys* **177**, L1-L2 (1987)

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Photoisomerization of OClO: a possible mechanism for polar ozone depletion

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CONNECTIONS between polar ozone depletion and halocarbon chemistry have been established by a number of studies[1-10] Recent attempts to account quantitatively for the observed rate of ozone decline in Antarctica in terms of known photochemical processes have not been entirely successful, and it seems that further chemical ozone-depleting mechanisms may be needed, particularly if the transport of ozone into the polar regions competes with chemical losses. Spectroscopic and photochemical data indicate that photolysis of OClO may provide a further ozone loss mechanism that has not previously been considered Here we report laboratory studies of OClO spectroscopy and photoproducts which suggest

that atomic Cl and O_2 are formed to some extent in the photodissociation process. This evidence points towards possible photoisomerization to the unstable species ClOO, (or at least to a similar metastable intermediate) probably by way of the ${}^2B_2$ excited state of OClO, thus reinforcing the idea that photolysis of OClO may contribute to polar ozone depletion

The discovery by Farman *et al*[1] in 1985 that a precipitous drop in Antarctic ozone had occurred has been the impetus for intensive studies of the Antarctic stratosphere Those studies quickly established that the Antarctic ozone decline occurs largely in September[2-4] when the total column of Antarctic ozone drops by ~50% as compared with historical values obtained in the 1960s A number of workers suggested that the decline in ozone might be related to increasing chlorofluorocarbon abundances[1], and noted that the unique depletion in the Antarctic spring could result from the combination of extremely cold temperatures which produce polar stratospheric clouds (PSCs), and the presence of sunlight to drive photochemical processes[5-7] Detailed studies of the stratospheric composition during September (in 1987, in particular) revealed that the chemistry of the Antarctic stratosphere had been greatly perturbed, leading to large abundances of chlorine radicals that can destroy ozone quite readily in the sunlit atmosphere[8,9] These studies provided firm links between the ozone loss and halocarbon chemistry

Although it is clear that the highly unusual chlorine chemistry induced by heterogeneous reactions occurring on the surfaces of polar stratospheric clouds can account for a substantial fraction of the ozone loss, it is difficult to be more quantitative because of uncertainties in both the laboratory and field measurements A number of catalytic cycles involving chlorine monoxide radicals participate in the chemical destruction of ozone in the sunlit Antarctic stratosphere Recent studies[8-11] are in general agreement that formation and photolysis of the ClO dimer is the dominant mechanism for Antarctic ozone destruction, at least during 1987 Coupling between chlorine and bromine represents a second important ozone loss pathway, the rate limiting step of which is

$$\mathrm{ClO + BrO \rightarrow Br + ClOO} \qquad (1)$$

Although reactions involving the ClO dimer are likely to dominate ozone loss in Antarctica under cold conditions and when ClO abundances are high, the bromine-chlorine mechanism assumes greater importance at warmer temperatures or when ClO enhancements are modest It is therefore likely to play a major and possibly dominant role in Arctic ozone loss, but also contributes ~20-30% of the Antarctic ozone depletion according to present understanding Barrett *et al*[8] suggested that the formation and photolysis of the ClO dimer could explain virtually all of the observed Antarctic ozone loss in 1987 based on their measurements of chlorine monoxide, but recent measurements for the reaction rate constant of ClO with itself to form the dimer (Cl_2O_2) have reduced the apparent importance of this mechanism There is now considerable question as to whether or not currently understood chemical mechanisms can explain the observed rate of Antarctic ozone loss[9-11] One study[9] estimated that only 65% of the total observed Antarctic ozone loss rate could be accounted for through the photochemistry suggested to date, whereas others conclude that the photochemic loss may be sufficient to account for the entire ozone decline

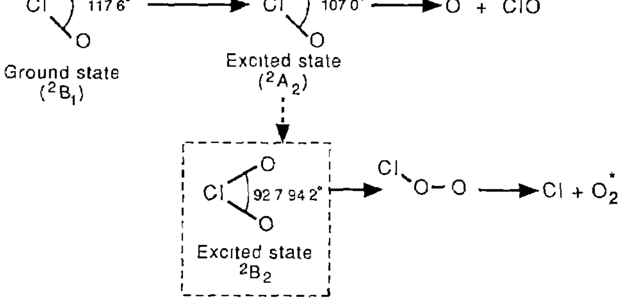

FIG 1 Schematic diagram of the suggested photochemistry of OClO

Many studies have implicitly or explicitly assumed that the Antarctic vortex acts entirely as a 'containment vessel' in which chemistry takes place in total isolation Any downward or poleward transport of ozone into the depleted region will probably require considerable increases in the rate of chemical destruction to account fully for the observed ozone decrease[12,13]

The interest in polar ozone has led to a fuller understanding of many of the chemical mechanisms that occur in the lowest part of the stratosphere In spite of this rapid progress, it seems likely that other unidentified chemical processes could contribute to the ozone decline In particular, it is widely accepted that a second product channel of the reaction between ClO and BrO leads to formation of OClO

$$\mathrm{ClO + BrO \rightarrow OClO + Br} \qquad (2)$$

This process is not expected to account for a net ozone loss, as rapid gas-phase photolysis of OClO has hitherto been assumed to produce exclusively atomic oxygen, which will reform ozone by the reaction with molecular oxygen

$$\mathrm{OClO} + h\nu \rightarrow \mathrm{ClO} + \mathrm{O}(^3\mathrm{P}) \qquad (3)$$

Note the contrast between the chemistry of OClO, which is thermally stable, and its isomer, ClOO, which is extremel unstable

If photolysis of OClO were to involve the following two-step process

$$\mathrm{OClO} + h\nu \rightarrow \mathrm{ClOO}\ \text{(photoisomerization)} \qquad (4)$$

$$\mathrm{ClOO + M \rightarrow Cl + O_2 + M} \qquad (5)$$

then formation of OClO would be a chemical pathway to ozone destruction If all OClO photolysis were to proceeed through photoisomerization, then the ozone loss rate that is due to the coupling of chlorine and bromine would roughly double (as the rate constants for reactions (1) and (2) are believed to be comparable at cold temperatures) Given the extremely labile nature of ClOO, it is expected to be very short lived and, probably cannot be observed directly following the photolysis of OClO in the gas phase However, the final outcome of the near-ultraviolet photolysis of OClO by reactions (4) and (5), namely formation of O_2 and Cl, is measurable in the laboratory and of importance in connecting OClO to the stratospheric ozone depletion Here we show that laboratory evidence indicates that OClO near-ultraviolet photolysis involves just such a photoisomerization mechanism We also discuss the spectroscopic and photochemical data available regarding the possible role of photoisomerization in the photochemistry of OClO

The spectroscopic information available can be used to obtain accurate excited-state structures and establish the compatibility of the proposed photochemistry with the observed changes in bond length and bond angle that occur on excitation Specifically, activity in the asymmetric bond stretch (ν_3 in the OClO spectrum) is needed to break a Cl-O bond Conversely, a decreased bond angle reflected in activity in the bending mode (ν_2) in the excited state, provides the geometry change required for photoisomerization

The near-ultraviolet spectrum of OClO extends from 21,000 to 37,000 cm^{-1} (270-476 nm), the observed features being attributed to a transition from the ${}^2B_1$ ground state to ${}^2A_2$ excited electronic state[14-16] In this transition[16 17], the Cl-O bond length increases from 1 47 Å in the ${}^2B_1$ state to 1 619 Å in the ${}^2A_2$ excited state, and the bond angle decreases substantially from 117 6° (${}^2B_1$) to 107 0° (${}^2A_2$) as shown in Fig 1

The excited-state structure and vibronic lifetimes are significantly perturbed by the efficient photochemistry occurring on excitation of OClO in the near-ultraviolet The photoreactivity at this energy is presumed to be linked to coupling of the $^2A_2$ state with the symmetry-forbidden $^2B_2$ state[17-19] The latter has eluded direct spectroscopic observation, but is calculated[17] to have a minimum bond angle ranging from 94 2° to 92 7°, and consequently to favour photoisomerization[17] (see Fig 1)

In principle, examination of a structured spectrum such as that of OClO can provide the desired information on the excited state At room temperature, however, spectra are too congested for such an analysis because of the thermal populations of many ground-state rotational levels, all of which are involved in the electronic transition As a result, the vibronic linewidths are dominated by thermal effects that exceed the natural linewidths and therefore preclude obtaining the excited-state lifetime

We have re-examined the spectroscopy and photochemistry of OClO free of inhomogeneous effects[20] by preparing samples in supersonic molecular jets The jet-cooled A $(^2A_2) \leftarrow X(^2B_1)$ absorption spectrum of OClO has recently[20] been obtained in our laboratory with an apparatus that combines a high-resolution Fourier Transform ultraviolet spectrometer with a high-throughput molecular jet Figure 2 shows a portion of the spectrum resulting from the transition to the $^2A_2$ state of OClO In the absence of thermal effects, the Heisenberg uncertainty principle establishes an inverse relationship between vibronic linewidths and excited-state lifetimes Thus the cold spectrum allows dynamical information to be obtained We can draw two conclusions from our spectra First, all vibronic linewidths increase with energy The corresponding lifetimes decrease from ~100 ps at 23,000 cm^{-1} (~435 nm) to ~1 ps at 33,000 cm^{-1} (~303 nm), reflecting the timescale of photochemical events Second, comparing the lifetimes of the three vibronic modes of OClO at approximately the same energy indicates that both the asymmetric stretch and the bend are involved in the photoreactivity of this molecule excited in the near-ultraviolet As a consequence of photodissociation, the asymmetric stretch displays very short lifetimes even at low energy, the $\nu_3 = 4$ level, for example, becomes so broad that it disappears into the noise (having a linewidth of 100 cm^{-1} at ~30,000 cm^{-1}) At the same time, progressions involving the bending mode (ν_2) broaden substantially at approximately the same energy, supporting the conjecture that ν_2 is involved in coupling the $^2A_2$ to the $^2B_2$ state, which is expected (in light of the small bond angle[17]) to favour photoisomerization

In summary, the spectroscopic evidence is consistent with both photodissociation and photoisomerization occurring on the near-ultraviolet excited electronic surface

The photochemistry of OClO has been investigated extensively using flash photolysis and laser techniques[22-25] Most authors agree that OClO dissociates[19,21-24] into ClO and O (reaction (2)), but some[19,24] have suggested alternative pathways (reactions (4) and (5)), leading to Cl and O_2^* Here, O_2^* could be, on the basis of energy, the $a^1\Delta_g$, the $b^1\Sigma_g$ excited states or vibrationally hot levels of the ground state $^3\Sigma_g$ To date, the primary photochemistry of OClO remains controversial, as experimental studies are complicated by rapid secondary reactions that obscure the primary events

To elucidate the primary photochemistry of OClO in the gas phase, we are reinvestigating this problem by preparing the molecule free of collisions in supersonic expansions The detection is based on resonance-enhanced multiphoton ionization/mass spectrometry (REMPI/MS) We measure strong, wavelength-dependent ClO^+ and Cl^+ mass signals following OClO photolysis Molecular oxygen has an extremely low multiphoton ionization (MPI) cross-section[26], making it difficult to detect in our system The Cl^+ action spectrum appears between 360 and 363 nm It is likely that this signal reflects the coupling of OClO and ClOO, or the REMPI structure of the isomerized parent molecule, ClOO REMPI of other precursors such as OClO or ClO can be excluded Our interpretation implies photoisomerization of OClO, followed by dissociation to produce atomic chlorine which is then probed by the MPI process This finding is consistent with Gole's analysis that isomerization of OClO occurs by the $^2B_2$ state (ref 17), as seen in the matrix experiment of Arkell and Schwager, most efficiently at ~365 nm (ref 27) The relative yield of the neutral photolysis products ClO, O_2 and Cl cannot be obtained from the data at this stage because of the unknown and very different MPI cross-sections which govern the signals detected These results suggest, however, that excitation in the near-ultraviolet of isolated OClO in the gas phase leads to both photodissociation to form ClO and to photoisomerization which ultimately leads to formation of O_2 and Cl

The involvement of photoisomerization is compelling in light of the matrix photochemistry[17 27,28] of OClO Photolysis of the symmetric chlorine dioxide in the near-ultraviolet electronic state, in argon, nitrogen or sulphuric-acid matrices, leads exclusively to formation of ClOO Quantum chemical calculations[17] explored the nature of the isomerization process and a mechanism was proposed for the concerted conversion of OClO to ClOO Photoisomerization is expected[17] to occur by coupling of the $^2A_2$ state to the $^2B_2$ state, as already mentioned (see Fig 1) The efficiency of this photochemical process is governed by both the position of the curve crossing between the two electronic surfaces and the magnitude of the coupling between them

We suggest a new model for the near-ultraviolet primary photochemistry of OClO vapour that is consistent with all the spectroscopic and photochemical data We propose that two photochemical reactions take place, namely photodissociation and photoisomerization, as shown in Fig 1 This model can explain the differences between the gas and condensed-phase photochemistry as it proposes that the relative quantum yields for photodissociation and photosiomerization vary with energy

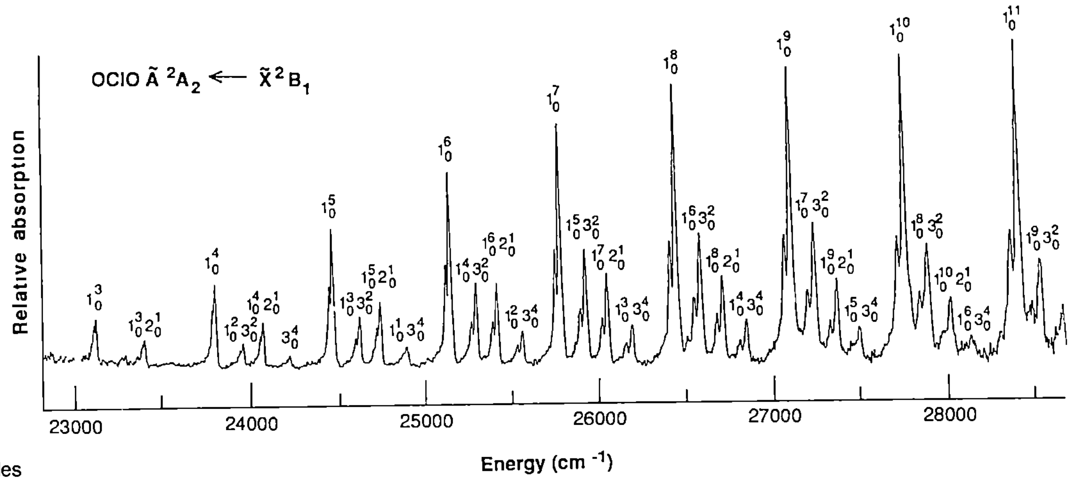

FIG 2 A portion of the jet-cooled absorption spectrum of the A←X transition of OClO, recorded at 5-cm^{-1} resolution under conditions of 3% OClO in He at a stagnation pressure of 2 atm The splitting in each band is caused by ^{35}Cl and ^{37}Cl isotopes All of the vibrational modes are observed in this spectrum The most intense progression has been assigned to excitation of the symmetric stretching mode (ν_1), whereas the less intense progressions involve the bending (ν_2) and the asymmetric stretching (ν_3) modes

Figure 1 shows the results from our measurements of axis ratio as a function of drop diameter d. For the smallest drops ($d \leq 1$ mm), our observations of axis ratios agree with the theoretical quiescent shape[7,9] within experimental uncertainty. The previous experimental data at small sizes[10] shows approximately the same scatter but seems to have a systematic error. In the present experiment, such errors were carefully evaluated and minimized using glass spheres to compare axis ratios obtained using the photographic method with those from direct measurements.

The measured axis ratios show a large two-sided scatter for $d \geq 1.4$ mm and a moderate one-sided scatter, above the theoretical curve, for $d \approx 1.0$–1.3 mm. The large two-sided scatter can be explained by axisymmetric mode oscillations of oblate- and prolate-like shapes having a two-sided axis-ratio variation, as Fig. 2 shows. Also, our measurements of the axes for the largest sizes revealed the characteristic opposing variation in v and h. The observed one-sided scatter for $d \approx 1.0$–1.3 mm is consistent with transverse-mode oscillations of the lowest harmonic. As Fig. 2 shows, the maximum vertical extent increases more than the maximum horizontal extent for various side views so that observations of v/h should yield a one-sided variation above the quiescent axis ratio.

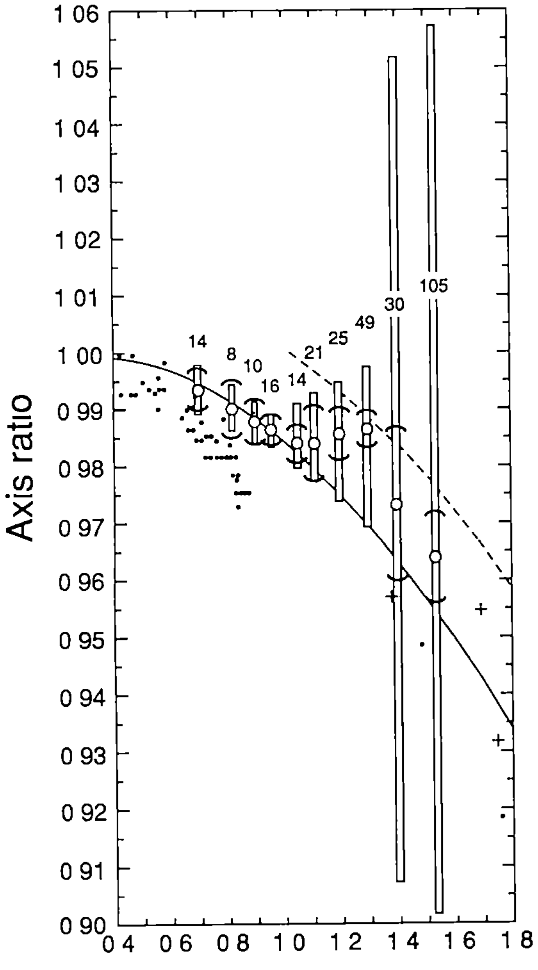

FIG. 1 Measured axis ratios as a function of drop diameter. The vertical bars show the range of measured values, including scatter and estimated (r.m.s.) experimental uncertainty. The uncertainty decreases with size at a fixed magnification because a measurement of axis ratio becomes more precise. Also displayed are mean axis ratios (circles), 95% confidence intervals (arcs) and number of measurements. The width of the bars indicates uncertainty from minor changes in drop size during (or between) experiments. About 4,000 photographs were needed to obtain 292 axis ratios, because of a preponderance of out-of-focus drops (horizontal scatter in fall trajectories was much larger than depth of field). The theoretical quiescent axis ratio is the solid curve[7-9] and previous wind-tunnel measurements are dots[10] and crosses[7]. The dashed curve shows the postulated shift in axis ratio based on polarization radar measurements in rain[6].

Further support for these postulated modes is the consistency of oscillation energies despite the different axis-ratio amplitudes in Fig. 1. Energies are comparable if the transverse mode is assumed for $d \approx 1.0$–1.3 mm and the axisymmetric mode is assumed for $d \geq 1.4$ mm (~0.2–0.4% of the surface energy). By contrast, energies differ by a factor of 20 if the reverse is assumed (axisymmetric mode at the smaller sizes and transverse mode at the larger sizes).

The onset of raindrop oscillations at $d \approx 1.0$ mm is close to the onset of eddy shedding for falling drops and spheres (Fig. 3). In addition, the observed onset of sideways drift also occurs just above $d = 1.0$ mm. For drops of $d = 1.0$–1.3 mm the match between oscillation and eddy frequencies is best at the lowest harmonic ($n = 2$). The two largest sizes have a better match with the two higher harmonics ($n = 3$ and 4). Although different harmonics have distinct shapes, it was difficult to distinguish among them from the drop photos because the single view of the profile could correspond to more than one mode, and the distortions were hard to identify at these small amplitudes.

Another distinguishing characteristic of each harmonic is its oscillation frequency, for example $n = 3$ has a frequency about twice that frequency of $n = 2$ (Fig. 3). Oscillation frequencies were measured by recording drop-fall streaks from a xenon light by using the modulation of the rainbow (Fig. 4). Large-amplitude oscillations produce dashed streaks in back-scattered light[14], as can be seen by shining a floodlight at rain or water dripping from a tree branch. Such streaks occur when the angle Ψ changes around the primary rainbow angle as light is deviated from the bow into the dark band. Our streak photos revealed dashes of spacing λ corresponding to the frequency of the fundamental harmonic f_2 ($f \approx f_2$), using the known terminal fall

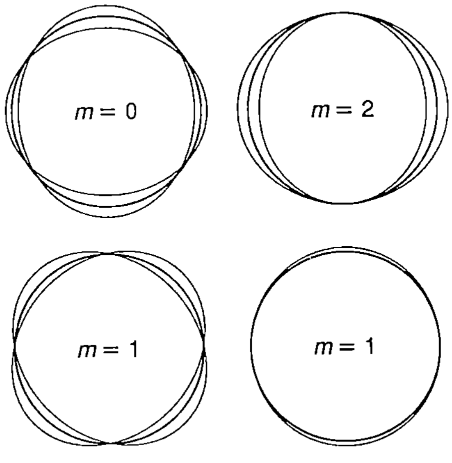

FIG. 2 Horizontal views (thin lines) for fundamental spherical harmonic ($n = 2$) and unperturbed spheres (thick lines). The three modes ($m = 0, 1, 2$) of equal oscillation energy (1% of surface energy) correspond to associated Legendre functions. The axisymmetric mode ($m = 0$) has oscillations of oblate- and prolate-like shapes with a two-sided axis-ratio variation about unity. The transverse mode ($m = 1$) is shown for two orientations (side views), 90° apart in the horizontal plane. The maximum vertical extent (v) increases more than the maximum horizontal extent (h) for various azimuthal views so that observations of v/h yield a one-sided variation above unity. The horizontal mode ($m = 2$) oscillates in the horizontal plane with major and minor axes 90° apart. The vertical axis remains fixed whereas the horizontal axis is larger or smaller than the vertical axis, producing axis ratios with a two-sided variation about unity. All three modes have been observed for water drops falling in air with oscillations about a quiescent raindrop shape rather than a sphere[20].

speed U and $f = U/\lambda$, with an average experimental uncertainty of only 3% in the measurement of f

The streak observations substantiate our conclusions based on the behaviour of the scatter in axis ratio small raindrops oscillate at the fundamental harmonic in the transverse and axisymmetric modes At the onset of eddy shedding the fundamental harmonic is excited, as might be expected from the match in frequencies (Fig 3) Oscillations occur in the transverse mode perhaps as a response to pressure changes caused by eddies detaching from alternate sides of the upper pole At larger sizes the oscillation response to eddy shedding is the axisymmetric mode of the fundamental harmonic, despite the poor match of $n = 2$ with the shedding frequencies[15-19]

The cause of the broad coupling between the fundamental mode and eddy shedding is unknown One possibility is that, once oscillations become large enough, shedding is triggered by shape changes, thereby shifting the shedding frequency to the oscillation frequency Another possibility is a subharmonic coupling of eddies to the oscillations If pressure patterns were alternatively in and out of phase with the distortion, this could explain why the transverse mode is not excited It is unclear how the forcing pattern would best match the axisymmetric mode or why the fundamental is preferred to the next higher harmonic

An indication that the observed strong-coupling phenomenon may extend to much larger raindrop sizes is apparent from the consistency of our axis ratios with those from the differential reflectivity, Z_{DR}, measurements in rain[6] (Fig 1) As Z_{DR} is proportional to the logarithm of the ratio of backscattered powers for radar pulses having vertical and horizontal polarizations, it is directly related to the axis ratio of raindrops The variation in axis ratio given by the dashed curve in Fig 1 was devised to rectify values of Z_{DR} measured with a high-resolution radar (Chilbolton, UK) with values calculated from observed raindrop size distributions This postulated shift in axis ratio, which was applied as an average effect from 1 to 3 mm diameter, is a likely consequence of raindrop oscillations promoted by eddy shedding, at least for small raindrops The occurrence of raindrop oscillations for $d = 2$–3 mm is also supported by recent airborne observations of raindrop shape[5] Such oscillations of moderate-size raindrops probably originate from collisions[11], but may persist because of intrinsic aerodynamic forces, for example from feedback of the varying aerodynamic pressure or drag, opposing the viscous damping of oscillations

To obtain further data on larger drops, we plan to extend our measurements of natural shapes up to 4 mm in diameter by using our experimental methods in a seven-story experiment A careful analysis of shape variations, oscillation amplitudes and oscillation frequencies should uncover more of the mysterious physics that underlies raindrop oscillations, and provide valuable information for understanding the scattering of microwave radiation in rain □

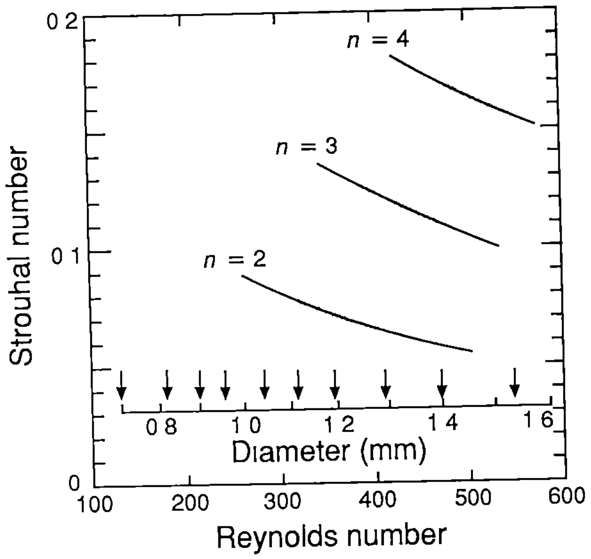

FIG 3 Dimensionless frequency (Strouhal number = fd/U) as a function of water-drop diameter and dimensionless flow parameter (Reynolds number = dU/viscosity) The oscillation frequencies for water drops in air are given by curves for harmonics $n=2, 3$ and 4 Previous measurements of eddy shedding frequencies are shown by the stippled area for drops[15,16] and rigid spheres[17] falling in liquids in a region with Strouhal number <0 15 and for supported spheres[18,19] in a region with Strouhal number >0 15 These data are for nonoscillating drops and rigid spheres The arrows show sizes for drops falling in air in the present experiment

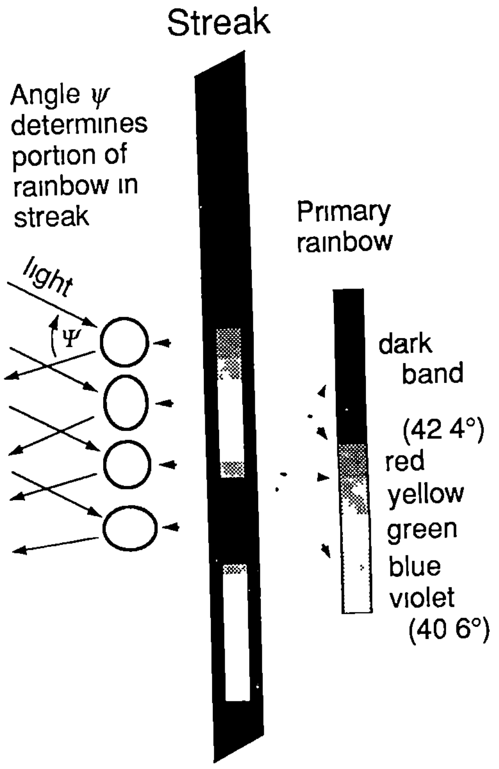

FIG 4 Schematic of modulated fall streak showing the associated drop oscillation and rainbow The oscillation frequency was measured from the spacing of dashes in photos of the fall streaks The dotted arrows show how portions of the fall streak correspond to the origin of colour in the rainbow, and how drop distortions yield a change in angle Ψ The angles are shown for red and violet Colours are weak, except near red, because the width of the light allows incident rays over a range of angles, thereby mixing the adjacent colours

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1 Seliga T A & Bringi V N *J appl Met* **15**, 69-76 (1976)
2 Hall M P M Cherry S M Goddard J W F & Kennedy G R *Nature* **285**, 195-198 (1980)
3 Illingworth A *Nature* **336**, 754-756 (1988)
4 Jones D M A *J Met* **16**, 504-510 (1959)
5 Chandrasekar V Cooper W A & Bringi V N *J atmos Sci* **45**, 1323-1333 (1988)
6 Goddard J W F & Cherry S M *Radio Sci* **19**, 201-208 (1984)
7 Pruppacher H R & Pitter R L *J atmos Sci* **28**, 86-94 (1971)
8 Beard K V & Chuang C *J atmos Sci* **44**, 1509-1524 (1987)
9 Beard K V Feng J Q & Chuang C *J atmos Sci* **46**, 2404-2418 (1989)
10 Pruppacher H R & Beard K V *Jl R Met Soc* **96**, 247-256 (1970)
11 Beard K V Johnson D B & Jameson A R *J atmos Sci* **40**, 455-462 (1983)
12 Gunn R *J geophys Res* **54**, 383-385 (1949)
13 Czys R R & Ochs H T *J atmos Sci* **45**, 3161-3168 (1988)
14 Blanchard D C *J atmos Sci* **19**, 119-120 (1962)
15 Magarvey R H & Bishop R L *Can J Phys* **39**, 1418-1423 (1961)
16 Magarvey R H & Bishop R L *Phys Fluids* **4**, 800-805 (1961)
17 Goldburg A & Florsheim B H *Phys Fluids* **9**, 45-50 (1966)
18 Achenbach E *J Fluid Mech* **62**, 209-221 (1974)
19 Moller W *Phys Z* **39**, 57-80 (1938)
20 Beard K V *J atmos Sci* **41**, 1765-1774 (1984)

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Simulation of the regional climatic impact of Amazon deforestation

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THE Amazon basin contains about half of Earth's Tropical forest[1]. Population pressure and subsequent demands for crop production, timber and firewood have led to rapid deforestation Quantitative estimates of the rate of deforestation from analysis of Landsat observations indicate that rates are increasing exponentially in many regions[2,3], but the precise figures are not known[4] Removal of the protection provided by natural cover can lead to soil erosion, disturbance of the ecosystem and reduction in species diversity[5] Here we report results from a three-year simulation, using a general circulation model, in which we replace Amazon tropical forest and savannah with pasture The simulated local climate response was dominated by a weakened hydrological cycle, with ess precipitation and evaporation and an increase in surface temperature. The reductions in precipitation and evaporation were mostly caused by changes in surface roughness and albedo: decreased roughness dominated the reduction in evaporation (and the increase in temperature), whereas the increased albedo was the main cause of a decrease in the moisture flux convergence (measured as the difference between precipitation and evaporation) contributing to the decrease in precipitation.

Within the Amazon basin, it is estimated that 50% of the rainfall is derived from local evaporation[6], and it has been suggested that a significant reduction in tropical forest might reduce evaporation and rainfall[7] Recently, various studies concerning the impact of surface changes on climate have been carried out using three-dimensional atmospheric global climate models (AGCMs) A review of tropical deforestation experiments[8] shows that substantial differences exist between the schemes for representing physical processes (parameterizations) and between methods used to simulate land transformations in AGCMs As a result AGCMs have not presented a uniform picture of the potential effects of deforestation on climate The use of rather simple land surface schemes and the lack of grid-scale data with which to calibrate the models make the applicability of the results uncertain Some common points, however, have emerged from these simulations First, albedo, roughness and surface hydrology are the most important variables for the representation of land transformation Second, results are sensitive to the parameterization processes in the model, to the choice of vegetation and soil parameters and the soil-moisture field as input data, and the climatology of the AGCM Over the past few years more realistic land surface parameterizations have been included in various AGCMs including that of the UK Meteorological Office

Here we describe experiments that were carried out using the $2\frac{1}{2}^\circ \times 3\frac{3}{4}^\circ$ resolution AGCM described in ref 9, but including a more complex representation of interactive cloud processes[10] The land surface parameterization (D A W , A B Sangster and A Slingo, unpublished results) includes a four-layer soil temperature scheme Interception of precipitation by a vegetation canopy is represented, allowing zero resistance to re-evaporation and potentially a more realistic partitioning of energy between latent- and sensible-heat fluxes The hydrological scheme diagnoses surface runoff from the excess of rainfall over infiltration capacity (accounting for the spatial variability of rainfall statistically), and subsurface runoff from the gravitational drainage of soil moisture[11] Geographical variation of land surface and soil types is based on the work of Wilson and Henderson-Sellers[12] Their original $1^\circ \times 1^\circ$-resolution primary- and secondary-vegetation datasets were used to create spatially varying datasets of 17 soil and vegetation parameters which were interpolated onto our $2\frac{1}{2}^\circ \times 3\frac{3}{4}^\circ$ model grid[13]

We ran three-year control and deforestation experiments, starting at the end of June All grid boxes in South America north of 30° S defined as either tropical forest or savannah on the $1^\circ \times 1^\circ$ grid were replaced by tropical pasture Soil characteristics except surface infiltration capacity, were left unaltered We give the results for an area in the southern part of Amazonia where the largest change in land surface characteristics occurred (see Fig 1)

We compare the control simulation with observation in Fig 1 and Table 1 The model simulates accurately the spatial distribution of rainfall for both the Southern Hemisphere winter and summer, although the dry areas are more extensive in winter over the southern Amazon and rainfall is slightly excessive in summer over the basin Looking particularly at the region concerned, Fig 2*a* shows this slight amplification of the seasonal cycle in the control rainfall simulation The annual mean values of other variables, compared in Table 1, show that the control simulation is quite realisitc We note that it is extremely difficult to obtain accurate estimates of such quantities because observations are too sparse to provide grid-square means Runoff is

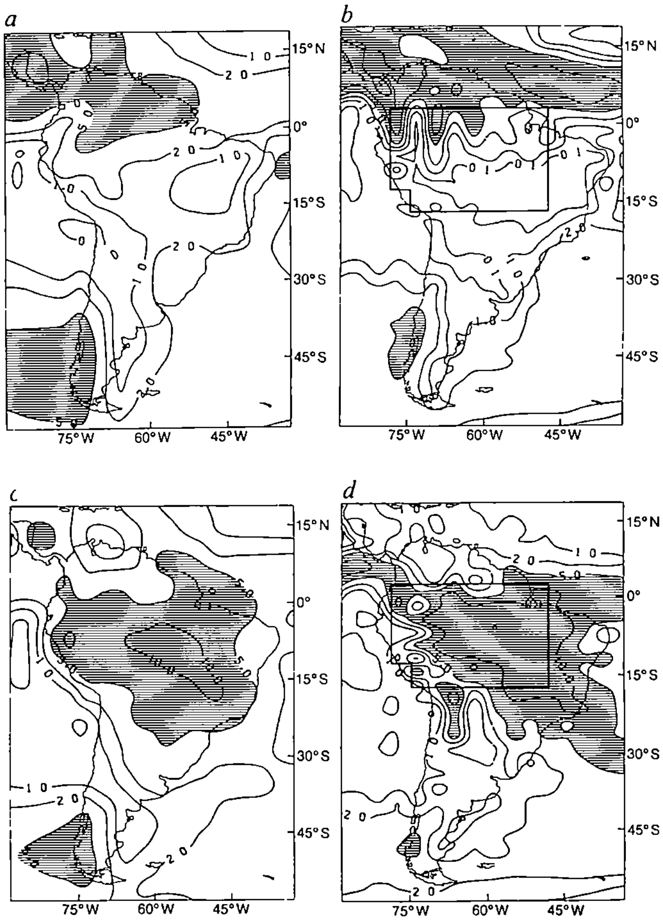

FIG 1 The section within the box shown in *b* and *d* represents the area selected for averaging purposes for all results quoted and represents the area having the largest change in land surface characteristics in the southern region north of 30° S *a*, Observed[21] precipitation pattern over South America for the Southern Hemisphere winter (June, July and August) Contours are at 0 1 1 0, 2 0, 5 0, 10 0 and 20 0 mm d^{-1} and shading is above 5 mm d^{-1} *b*, Simulated precipitation pattern of the control experiment over South America for the Southern Hemisphere winter averaged over three years *c*, As for *a* but for the Southern Hemisphere summer (December, January and February) *d*, As for *b* but for the Southern Hemisphere summer

TABLE 1 Summary of surface variables

Surface Variable	C*	D†	O‡
Evaporation (mm d^{-1})	3.12	2.27 (−27.2%)	3.34 (ref. 21)
Precipitation (mm d^{-1})	6.60	5.26 (−20.3%)	5.26 (ref. 22)
Soil moisture (cm)	16.13	6.66 (−58.7%)	
Runoff (mm d^{-1})	3.40	3.0 (−11.9%)	2.76 (ref. 23)
Net radiation (W m^{-2})	147.3	126.0 (−14.5%)	
Temperature (°C	23.6	26.0 (+2.4 °C)	24.0 (ref. 24)
Sensible heat (W m^{-2})	57.2	60.2 (+5.2%)	
Bowen Ratio	0.85	1.5 (+76.5%)	

* Control simulation, averaged over three years

† Deforested simulation. averaged over three years

‡ Observations

only quoted for a particular basin and is not strictly comparable to that from the region shown. Evaporation is based on only a few direct observations and empirical calculations.

Comparison of surface quantities for a single model grid square with data[14] for a site in central Amazonia indicates that modelled total evaporation differs by 15% from the observed, and that the modelled canopy evaporation is 71% compared with the observed 25% of total evaporation. Overestimation of canopy evaporation is probably present in other land surface schemes and this may be due[15] to the extension of a single-point description of the rainfall interception process to the grid-scale area in a region where convective rainfall events dominate.

Table 1 also summarizes the effect of deforestation on local climate for a variety of surface variables from each experiment. The hydrological cycle is significantly weakened by deforestation, with a reduction in evaporation, rainfall and runoff. Surface runoff is reduced as the decrease in intensity and frequency of rainfall events predominates over an opposing tendency for increased runoff that is the result of a reduction in infiltration capacity. The decrease in available soil moisture occurs as a result of the marked reduction in root depth (from 127.4 cm to 61.9 cm). Pasture reflects more solar radiation than does forest, which is a very efficient absorber and scatterer of short-wavelength radiation. Consequently, the net energy absorbed by the surface was diminished, with the mean albedo increased from 0.136 to 0.188. By itself, a reduction in net absorption of radiation at the surface would result in a temperature decrease but this is offset by a reduction in evaporative cooling and so there is an overall rise in temperature. The decrease in the latent-heat flux was due to the reduction in roughness (from 0.79 m to 0.04 m) and, at some times of the year, the water availability, this decrease is primarily responsible for the marked increase in the Bowen ratio (defined as the ratio of sensible to latent heat).

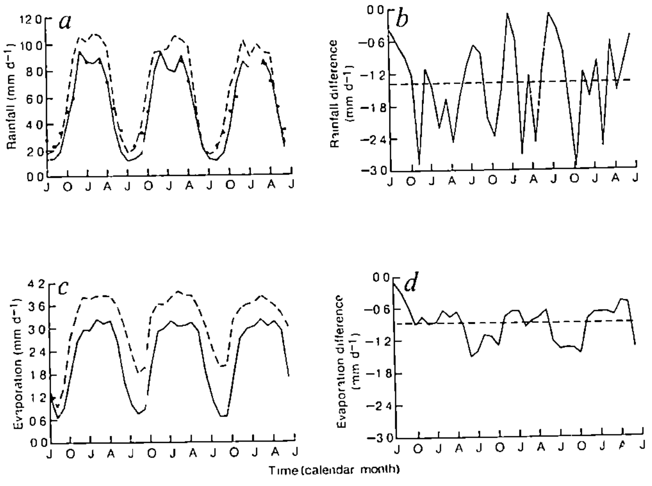

FIG. 2 *a*, Monthly mean precipitation (mm d^{-1}) averaged over the region under consideration for three years starting in July. The broken line represents the control simulation (C), the solid line the deforested simulation (D) and the dots the observed rainfall (O) (ref. 21). *b*, Simulated monthly mean difference (D − C) of precipitation (mm d^{-1}) averaged over the region under consideration for three years starting in July. The broken line represents the mean difference precipitation. *c*, As for *a* but for evaporation (mm d^{-1}). *d*, as for *b* but for evaporation (mm d^{-1}).

Figure 2 depicts the seasonal variation of precipitation and evaporation for the control simulation, the 'deforested' simulation and the difference between the two. There is a consistent decrease in precipitation and evaporation throughout the year although rainfall changes exhibit greater variability. There is also a seasonal variation in the change in evaporation with the maximum differences occurring in the winter and spring when evaporation is controlled by the limited availability of soil moisture.

To gain a better understanding of the physical mechanisms that are operating, two eight-month simulations, using the same initial data as the control and deforested experiments, were carried out to investigate the relative impact that is due to changes to albedo and roughness alone. Table 2 summarizes the differences between some of the surface variables of the control simulation and those of the experiments with increased albedo (from 0.136 to 0.188), decreased roughness (from 0.79 m to 0.04 m), and total deforestation. Precipitation within the Amazon basin originates from two sources—from the recycling of water vapour released during evapotranspiration, and from the moisture flux convergence into this area. The decrease in moisture flux convergence (measured as the difference between precipitation and evaporation) is due mainly to increased albedo although there is some contribution from the change in roughness. Increased albedo reduces the heat source, weakening ascent and boundary-layer convergence[16]. Reduced roughness can also decrease the moisture flux convergence as it weakens the boundary-layer convergence (associated with frictional drag near the surface) into the continental surface-pressure low[17]. Decreased roughness dominates the reduction in evaporation and the associated temperature increase. The forest canopy is wet for most of the year (implying low surface resistance) and so a decrease in evaporation is to be expected as roughness decreases[18]. Increased albedo reduces the available energy for upward turbulent transfer of latent energy. Hence, changes in both albedo and roughness make a major contribution to the overall decreases in precipitation and evaporation in the deforestation experiment.

Our results can be compared with a 13-month simulation carried out by Dickinson and Henderson-Sellers[19]. They incorporated a land surface scheme with a canopy in the National Center for Atmospheric Research Community Climate Model which has a grid resolution of 4.5°×7.5°. They assumed that all of the Amazon tropical forest in South America was replaced by impoverished grassland. Both experiments show a decrease in evaporation (~16 mm $month^{-1}$ in ref. 19 (from their Fig. 11) compared with 26 mm $month^{-1}$ here) and an increase in surface temperature (with a range of 2.0–5.0 °C over the 13 months in ref. 19 compared with the average of 2.4 °C here). Dickinson

TABLE 2 Summary of the differences in surface variables

Surface Quantity	A − C*	R − C†	D − C‡
Evaporation (E) (mm d^{-1})	−0.20	−0.43	−0.61
Precipitation (P) (mm d^{-1})	−0.75	−0.69	−1.34
$P - E$ (mm d^{-1})	−0.55	−0.26	−0.73
Temperature (°C)	−0.10	2.24	1.98

These results are averaged over eight months.

* A, increased albedo simulation, C, control simulation.

† R, decreased roughness simulation.

‡ D, 'deforested' simulation.

and Henderson-Sellers, however, found no detectable change in rainfall, in contrast to the average decrease of 1 3 mm d^{-1} (20%) shown here

Our results indicate a stronger impact of deforestation on the local climate than do previous GCM experiments We note, however, that these results are highly sensitive to the formulations of the model and our ability to make firm predictions based on these results is inhibited by the lack of observations in forested and deforested areas with which to verify these schemes More ground-based measurements of atmospheric and hydrological processes, together with global data sets of surface vegetation types derived from satellite data, could aid in this development, both as input to the model and to validate and adjust the land surface parameterizations[20] Nevertheless, our results indicate that deforestation can cause significant local climatic perturbations and further efforts should be made to improve our understanding of the processes involved ☐

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1 Dickinson R E (ed) *The Geophysiology of Amazonia* 3-10 (Wiley New York 1987)
2 Fearnside P M in *The Geophysiology of Amazonia* (ed Dickinson R E) 37-61 (Wiley New York 1987)
3 Malingreau J P & Tucker C J *Ambio* **17**, 49-55 (1988)
4 Neto R B *Nature* **339**, 86 (1989)
5 Lavelle P in *The Geophysiology of Amazonia* (ed Dickinson R E) 175-223 (Wiley New York 1987)
6 Salati E Marques J & Molion L C B *Interciencia* **3**, 200-205 (1978)
7 Salati E in *The Geophysiology of Amazonia* (ed Dickinson R E) 273-296 (Wiley New York 1987)
8 Henderson Sellers A in *The Geophysiology of Amazonia* (ed Dickinson R E) 463-496 (Wiley New York 1987)
9 Slingo A Wilderspin R C & Smith R N B *J geophys Res* **94**, 2281-2301 (1989)
Smith R N B *Q Jl R met Soc* (in the press)
Warrilow D A in *Proc Int Satellite Land-Surface Climatology Proj* 159-166 (European Space Agency 1986)
12 Wilson M F & Henderson-Sellers A *J Clim* **5**, 119-143 (1985)
13 Warrilow D A & Buckley E *Annls Geophys* **7**, 439-450 (1989)
14 Shuttleworth W J *Proc R Soc* **B233** 321-346 (1988)
15 Shuttleworth W J & Dickinson R E *Q Jl R met Soc* **115**, 1177-1179 (1989)
16 Charney J G *Q Jl R met Soc* **101**, 193-202 (1975)
17 Sud Y C Shukla J & Mintz Y *J appl Met* **27**, 1036-1054 (1988)
18 Rowntree P R *UN Univ Workshop Forests Climate and Hydrology—Regional Impacts* (United Nations University Tokyo 1984)
19 Dickinson R E & Henderson-Sellers A *Q Jl R met. Soc* **114**, 439-462 (1988)
20 *Int. Satellite Land-Surface Climatology Proj Report No* 10 (eds Becker F Bolle H J & Rowntree P R) (Free University of Berlin 1987)
21 Willmott C J Rowe C M & Mintz Y *J Clim* **5**, 589-606 (1985)
22 Jaeger L *Monatskarten Des Niederschlags Fur Die Ganze Erde Bericht Deutsche Wetterdienst* **18**, no 139 (1976)
23 *Discharge of Selected Rivers of the World* Vol 2 (UNESCO Paris 1971)
24 Schutz C & Gates W L *Report for Advanced Research Projects Agency Global Climatic Data for Surface 800 mb 400 mb January* (1971) *July* (1972) *April* (1973) *October* (1974) (Rand Santa Monica)

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Climate patterns revealed by pollen and oxygen isotope records of a Tyrrhenian sea core

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THE analysis of pollen from marine cores has produced continental palaeoclimate records which have been directly correlated with the oxygen isotope record of global ice volume and regional climate[1-3] Here we tie the palaeoclimate of southern Europe to this global climate signal by reporting continuous pollen and δ^{18}O records of a well-dated core in the Tyrrhenian Sea for the time span of 55–9 kyr BP These records show a strong covariation between the marine δ^{18}O reversed curve and a good terrestrial climate indicator, namely the pollen abundance of deciduous oak from the upland sub-humid Mediterranean forest of southern Italy and Sicily, which seems mainly constrained by moisture variation The *Artemisia* and grass pollen abundances document the shift between continental semi-desert and oceanic steppe climates Repetitive successions between 55 and 33 kyr BP of grass, oak, *Artemisia* and *Abies* pollen abundance peaks occur in locked phase with δ^{18}O depletion events and indicate that regional vegetation cycles closely accompany deglacial pulses of global extent

The stratigraphy of core KET 8003 (38°49 2′ N, 14°29 5′ E, 1,900 m water depth, 1,030 cm length), is based on the oxygen isotope variation in the foraminifer *Globigerina bulloides*, measured at 10-cm intervals[4] (Fig 1) Tests of calcite formed by marine organisms reflect the fluctuations of ^{18}O in sea water Because ^{16}O evaporates more readily than ^{18}O, the ^{18}O/^{16}O ratio is low in continental ice sheets and high in the glacial ocean Conversely, during interglacial periods, the oceans are depleted in ^{18}O The basis of this core lies within the last Interglacial, that is isotope stage 5e at 125 kyr BP The chronology is based on six interbedded ash layers that have been correlated by their chemical composition with lava flows in southern Italy[4,5] that were dated by ^{14}C and K/Ar analysis to be between 55 and 12 kyr old, and on the initiation of the deglaciation after the last glacial maximum (Termination I), globally seen at 15 kyr BP[6] Because the sample set for pollen was taken subsequently, it has a 2-cm offset from the isotope set Pollen has not been recovered below 580 cm

Southern Italy was also the source area for pollen in core KET 8003 both volcanic ash and pollen are airborne We have identified 128 pollen taxa and excluded *Pinus* from the pollen sum The large production and strong resistance of *Pinus* pollen to aerobic bacterial degradation, probably linked to its high sporopollenin content[7], often result in over-representation in marine cores[8] Here we describe the variation of the pollen percentages of *Quercus*, *Abies*, *Populus*, Poaceae, *Artemisia*, Chenopodiaceae and *Ephedra* A heavy pollen producer, *Quercus* (oak) is the only deciduous tree whose pollen is continuously present in the core Oak pollen percentage in core KET 8003 shows no correlation with (1) the pollen sum, (2) total pollen concentration per gram of sediment and *Pinus* pollen abundance These two observations indicate respectively that the percentage is not an artefact of the analysis procedure nor is it controlled by differential pollen preservation before and during burial on the sea floor

The δ^{18}O and oak pollen abundance series of core KET 8003 co-vary in phase with each other The peaks in oak pollen abundance usually just precede those of δ^{18}O depletion This, however, is an artefact of the sampling offset and does not preclude the existence of coincident peaks The partitioning of the records into two types of intervals, one of δ^{18}O decrease/oak pollen increase (climate improvement) and the other with the reverse relationship (climate deterioration) yields a high correlation between the two data sets during the former intervals ($r = 0$ 84), and a lower one during the latter ($r = 0$ 55)

During isotope stage 3, characterized by generally high δ^{18}O values which denote globally glacial conditions between 69 and 24 kyr BP, there are four closely matched events of δ^{18}O depletions and oak pollen peaks between 580 and 240 cm which can be seen as short-lived deglacial pulses Each of the two oldest peaks in oak pollen abundance, dated before 50 kyr BP, is set between two other abundance peaks an earlier one of Poaceae and a later (and larger) one of *Artemisia* This repetitive succession amounts to a pattern also seen later, during the first half of the deglaciation after 15 kyr BP Whereas the abundance of oak pollen decreases above 472 cm, that of *Abies* pollen increases strongly between 450 cm and 400 cm, and *Hedera* and *Ilex* pollen appear sporadically We suggest a correlation with the peaks of *Abies* pollen seen between 2,200 and 2,045 cm in the record of Lake Monticchio in southern Italy[9] After 33 kyr BP, *Quercus* and *Artemisia* pollen abundances become minimal and Poaceae is the prevailing herbaceous pollen taxon

During the glacial maximum (240–190 cm), corresponding to part of isotope stage 2 (24–11 kyr BP), the average abundance of oak pollen is at its lowest Until mid-stage 2, the dominant the uplands, *Abies* from the cool humid montane forest A ense, humid grass steppe is seen today from Hungary through e Ukraine (annual precipitation 350-455 mm) along 45-50° N, northern Kazakhstan at 50-55° N (ref 19) With a thick snow over providing ample spring moisture, the Ukrainian climate partly oceanic, and with a precipitation peak in summer, it partly continental The late summer drought eliminates eciduous trees, leaving the steppe a residual vegetation formaon The lowland semi-desert seen today in Middle Asia along 0° N is more arid (80-280 mm precipitation) and more connental (21-24 °C in July, −12 to −16 °C in January) than the krainian grass steppe Forests of deciduous trees below *Abies* orests today grow on the northern and western slopes of the ian-Shan mountains above the desert lowland that lies south f the semi-desert

We interpret the vegetation that produced the pollen record rom stage 3 as repeatedly shifting, in the lowlands, from semiesert to steppe, with a predominance of the former under a ghly continental climate The succession of abundance peaks or oak pollen indicates that pulses of moisture and temperature ncrease occurred in the uplands, followed by episodes of still et but colder climate, signalled by the high abundance of *Abies* ollen The rapid rise in oak pollen abundance after each glacial nterval indicates the ability of that tree to take advantage of he earliest moisture increase in a continental climate This can e accounted for by the 'hardening' process the survival of ormant buds is improved by low temperature (−25 °C and even -30 °C) when it occurs in January and February[19]

The scarcity of oak pollen during the last glacial maximum ndicates that pollen production decreased and/or that source reas were very restricted Isolated refuges of deciduous temperte forest have been hypothesized in the mountains of southern Europe, low enough to avoid extreme autumn cold, fatal to the ormant buds, and high enough to provide adequate summer oisture by adiabatic cooling of ascending air[13,14 20 21] The eplacement of the present Mediterranean vegetation of southern Italy at sea level by a grass steppe of Ukrainian type would result from a temperature decrease of 1-4 °C in July, to 24-21 °C, and of 10-24 °C in January, to 0 to −14 °C, and a 440-540-mm decrease in annual precipitation, to 450-350 mm Provided that precipitation would be ⩾500-600 mm, then a refuge zone for deciduous trees could exist up to 600 m, where temperature would be 17 4-20 4 °C in July, −3 6 to −17 6 °C in January (with a 0 6 °C per 100-m pseudoadiabatic lapse rate[22]) These temperatures compare well with those at the northern and north-east limits of the deciduous oak distribution in eastern Europe[19] Thus it might be that the lower temperatures during lacial times would not have restricted or eliminated the oak, whereas moisture decrease could have, as also suggested by estimates of glacial climate in Greece[14]

A modern analogue for the vegetation that produced maximum *Populus* pollen abundance at the onset of deglaciation in core KET 8003 is the aspen trees, which in the eastern Ukrainian steppe surround each small ground depression occupied by a rain-fed round lake (called a 'pod')[19] Although higher than in the treeless steppe, precipitation (~480 mm) is still not sufficient to develop a drainage system

During the early part of deglaciation, ~14 kyr BP while the upland oak population increased in the Tyrrhenian basin, the lowland oceanic grass steppe of the glacial maximum progressively gave way to a continental semi-desert, most widespread during the Younger Dryas

Reconstruction of the precipitation variation in Les Echets in south-east France[23] (Fig 2) can be compared with our oak pollen per cent variation In the two time-series, during stage 3, moderately high values are seen from 60 to 43 kyr BP, and low values at ~40 kyr BP, increasing during the last part of stage 3 Stage 2 is dryer and oak values are minimal, precipitation starts to increase at 15 kyr BP, as to the deciduous trees

The climatic significance of this isotope record is complex The 1 3‰ isotope effect of the global ice volume[24] accounts for 54% of the 2 40‰ δ^{18}O range between glacial maximum and the core top The 1 1‰ remainder is due to regional sea surface temperature and salinity variations As the isotope and oak pollen variations have comparable amplitudes in the strong deglacial pulse of early stage 3 from 560-542 to 472-466 cm depth (Δ (δ^{18}O) = 1 48‰, Δ oak pollen = 14%) and in the transition from glacial maximum at 190 cm to Allerod at 110-120 cm (Termination Ia) ($\Delta\delta^{18}$O = 1 37‰, Δ oak pollen = 16%), it is conceivable that the isotope shift of stage 3 could record an ice-volume decrease comparable with that of the first phase of deglaciation This implication, which requires that the range of salinity variation is also similar, is based on the present link between the formation or rejuvenation of atmospheric depressions, generating the regional precipitation to which the oak population is sensitive, and the high Tyrrhenian sea surface temperature[25,26]

Comparison of the course of events after the deglacial pulses of stage 3 and Termination Ia shows that the reversal to glacial conditions in the isotopic composition of the sea and in the vegetation of southern Italy was complete after 49 kyr BP, and partial after 11 kyr BP during the Younger Dryas We hypothesize that this difference might have some link with the observations that (1) the July insolation peak at 56 kyr BP was lower than that at 11 kyr BP (at 60° N, respectively 7 and 10% higher than present[27]) and (2) the rate of insolation increase for July was smaller at the transition from full-glacial stage 4 to stage 3 than at that from full-glacial stage 2 to stage 1 (at 60° N, respectively 7 2 cal cm^{-2} per 1,000 yr between 70 and 56 kyr BP, and 8 5 cal cm^{-2} per 1,000 yr between 22 and 10 kyr BP) The Termination II deglaciation between 1,350 and 1,250 kyr BP does not, to present knowledge, record a reversal similar to the Younger

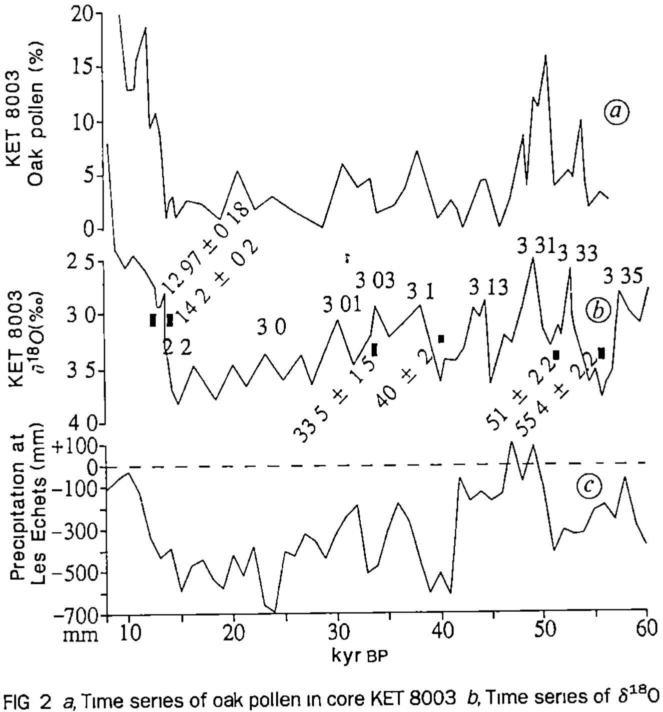

FIG 2 *a*, Time series of oak pollen in core KET 8003 *b*, Time series of δ^{18}O in core KET 8003 Chronostratigraphy based on radiometrically dated tephra (black rectangles) and the initiation of deglaciation at 15 kyr BP, isotope event 2 2 Event 3 0, the stage 2/3 boundary, is set at 24 kyr BP Isotope events after ref 5 *c*, Reconstruction of variation in annual total precipitation expressed as deviation from the modern value (800 mm) in Les Echets, southeastern France[23] Error bars omitted for simplification On comparing this with the KET 8003 time series, a lack of radiometric ages in Les Echets before 25 kyr BP should be noted The major precipitation peak in Les Echets at 49-46 kyr is synchronous with the peak in *Abies* pollen abundance in KET 8003

Dryas[28] The link of Termination II with a higher insolation peak and more rapid pace of insolation increase is consistent with the steering of the climate system

Broecker hypothesized that it is the shut down of the Atlantic Ocean conveyor belt that brought about the cooling trend in the climate jumps after 11 kyr BP and during stage 3 (refs 29 and 30) If this is the case, the heat that is no longer transported from low to high latitudes by ocean surface currents would accumulate in the tropical ocean There it would increase the convective activity of the Intertropical Convergence Zone, which would reactivate the atmospheric Hadley cell[31,32] and restore the poleward oceanic heat transport Thus the conveyor belt closing-off is a self-limiting process which can only be temporary

Inclusion in rapidly accumulating marine sediments of tl pollen originating in all elevations in the rimlands of the Ty rhenian Sea is a process that provides an integrated picture the vegetation through its rapid changes The complex patte of these changes documents the palaeoclimate of southe Europe during a timespan that is here particularly well date The covariation of the oak pollen abundance with the isotop composition of sea water, which is shaped in part by the glob volume of the ice sheets, testifies to the coherence of the fluct ations of the climate system at the millennial timescale in regional and global expressions

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1 Heusser L & Shackleton N J *Science* **204**, 837-839 (1979)
2 Van Campo E Duplessy J C & Rossignol-Strick M *Nature* **269**, 56-59 (1983)
3 Turon J L *Nature* **309**, 673-676 (1984)
4 Paterne M Guichard F Labeyrie J Gillot P Y & Duplessy J C *Mar Geol* **72**, 259-285 (1986)
5 Paterne M Guichard F & Labeyrie J *J Volcan geotherm Res* **34**, 153-172 (1988)
6 Bard E *et al Nature* **328**, 791-794 (1987)
7 Shaw G in *Sporopollenin* (eds Brooks J Grant P R Muir M D & Van Gijzel P) 305-348 (Academic London 1971)
8 Duplessy J C Delibrias, G Turon J L Pujol C & Duprat J *Palaeogeogr Palaeoclimatol Palaeoecol* **35**, 121-144 (1981)
9 Watts W A *J geol Soc Lond* **142**, 491-499 (1985)
10 Wijmstra T A *Acta bot neerl* **18**, 511-527 (1969)
11 Cwynar L C & Watts W A *Quat Res* **31**, 377-380 (1989)
12 Behre K E *Pollen Spores* **23**, 225-245 (1981)
13 Smit A & Wijmstra, T A *Acta bot neerl* **19**, 867-876 (1970)
14 Sabatier R & Van Campo M *Bull Soc bot Fr* **131**, 85-96 (1984)
15 Planchais N *Pollen Spores* **4**, 87-93 (1962)
16 Smit A *Acta bot. neerl* **22**, 655-665 (1973)
17 Bonin G & Gamisans J *Doc Phytos* **19-20**, 73-88 (1976)
18 Quézel P *UNESCO Note techn MAB* 2 9-33 (1976)
19 Walter H *Die Vegetation Osteuropas Nord- und Zentralasiens* (Fischer Stuttgart 1974)
20 Beug H J *Ber dt bot Ges* **80**, 682-689 (1967)
21 Van Campo, M in *Etudes françaises sur le Quaternaire* (Suppl *Bull AFEQ* Paris) 104-111 (196
22 Holton J R *An Introduction to Dynamic Meteorology* (Academic New York 1979)
23 Guiot J Pons A de Beaulieu J L & Reille M *Nature* **338**, 309-313 (1989)
24 Ruddiman W F in *North American and Adjacent Oceans During the Last Deglaciation* Vol (eds Ruddiman W F & Wright H E Jr) 463-478 (Geol Soc Am Boulder 1987)
25 Trewartha G T *The Earth s Problem Climates* (University of Wisconsin Press Madison 196
26 Gat J R & Carmi I *J geophys Res* **75**, 3039-3048 1970
27 Berger A *Quat Res* **9**, 139-167 (1978)
28 Woillard G M *Quat Res* **9**, 1-21 (1978)
29 Broecker W S Peteet D M & Rind D *Nature* **315**, 21-26 (1985)
30 Broecker W S *et al Paleoceanography* **3**, 1-19 (1988)
31 Bjerknes J *Tellus* **18**, 820-829 (1966)
32 Rowntree P R *Q Jl R met Soc* **98**, 290-321 (1972)

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Seismic reflection profiles across deep continental crust exposed in the Kapuskasing uplift structure

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SEISMIC imaging of Precambrian cratons holds the promise of deep structural mapping and interpretation of fundamental crustal construction processes Deep structures may be identified by tracing reflections to the surface[1], particularly in exposed crustal cross-sections[2] elevated along deeply penetrating faults, but many such cross-sections have structural orientations too steep for successful reflection imaging (like the Ivrea zone[3]) or occur in plate-boundary settings not representative of continental interiors By contrast, the Archaean greenstone terrain exposed in the Kapuskasing uplift of Canada[4,5] has gently dipping structures, and an intracratonic setting within the Superior Province, thus providing an opportunity to examine the third dimension of continental crust down to 25–30 km palaeodepth[6] Here we present new seismic reflection data which, although generally supportive of structural models based on surface geology[4], gravity modelling[5] and seismic-refraction studies[7,8], also indicate that the Kapuskasing structure is a relatively thin thrust sheet containing seismically reflective sequences of high-grade rock which may be analogues of seismically reflective *in situ* lower crust.

Superior Province, the largest preserved Archaean craton, is well known for its greenstone belts, hosting major gold an base-metal deposits The east-trending Abitibi–Wawa belt (Fi 1) of low-grade metavolcanic and granitoid rocks is transecte by the north-east-striking Kapuskasing uplift (KU), distin guished by high-grade metamorphic rocks producing positiv gravity and aeromagnetic anomalies[9] The KU is interpreted a a slab of deep crust[4,10,11], thrust upward along the west-dippin Ivanhoe Lake fault zone (ILFZ) A 120-km transect across th KU from Lake Superior to the ILFZ crosses greenstones wit 2–3 kbar metamorphic palaeopressures[12], amphibolite-facie (5–6 kbar) tonalite of the Wawa gneiss terrane, a density[13]-velocity[14] boundary inferred to be a Conrad-type discontinuity and heterogeneous, layered, high-grade (7–9 kbar) gneisses o predominantly magmatic origin in the Kapuskasing zone Increases in metamorphic grade and pressure across the K indicate an easterly deepening erosion level, from regional background values of 6–9 km in the greenstones to 25–30 km nea the ILFZ With increasing palaeodepth, decreasing U–Pb, Ar–A and Rb–Sr ages show that rocks from deeper levels remaine hot for longer and cooled more slowly[15] The crust attained mos of its 40 ± 5-km thickness in the interval 2 75–2 68 Gyr, throug early arc volcanism and plutonism, followed by shortening metamorphism and renewed arc magmatism[5] Seismic refractio results map a zone of high velocity (6 6 km s^{-1}) extending fron near the surface in the Kapuskasing zone to normal depths o ~20 km under the greenstones to the west[7,8] The crust thicken from regional values of 35–44 km to >50 km beneath the Kapuskasing zone

Since 1984 LITHOPROBE has supported a wide variety o geological and geophysical studies aimed at defining crustal-scale geometry and evolution through the Kapuskasing 'window' A key component of these studies is the 1987–88 seism reflection survey, three lines, labelled 1, 2–3–4 and 6, crosse the southern KU (Figs 1 and 2) Line 1 extended south to north from the Abitibi belt into the Kapuskasing zone Composite line 2–3–4, comprising three conventional segments and two undershoots regions containing reflection points but having no surface reflection sources or receivers, crossed the Kapuskasing zone cluding the Shawmere anorthosite complex parallel to the gional east–west structural grain In addition to the relatively onventional regional survey, line 2 was resurveyed using highsolution parameters, which yielded significantly more detail bout the shallow structure (compare Fig 3*a* and *b*) Finally, ne 6 sampled the southern end of the KU

The stacked seismic sections (Figs 2, 3) show many coherent vents although the image quality varies along the profiles pper crustal reflectivity is generally high along all lines, but e reflection character varies with the geological terrane Porons of the seismic lines that cross tonalitic gneiss and granite f the Abitibi belt yield prominent reflections, dipping west and orth at >30°, from near the surface to at least 13 s beneath the rface exposure of the KU The uppermost zone of strong eflectivity on lines 1 and 6 (H on Fig 2*a*, *c*) projects 10 km eyond the south end of the lines into outcrops of felsic gneiss nd 'homogeneous' granite

Reflectivity of the KU granulites and the seismic image of e ILFZ are best represented on line 2-3-4 Within the Kapusasing zone, shallow (0 2–2 s) 'E' reflections (Fig 3*b*) dip 15° W along the profile and can be traced short distances to urface exposures of mafic, tonalitic and anorthositic rocks terlayered on a 10–30-m scale[16] These sill-like intrusive bodies ave seismic volocities (7 23, 6 55, 7 26 km s^{-1} respectively)[17] nd dimensions appropriate to generate significant reflections lthough direct correlation of reflections and lithology would equire short drill holes[18] The basal reflection of the E package ', Fig 2*b*) locally truncates events above and below it, suggestg hanging-wall and footwall cutoffs by a splay of the ILFZ[19] pair of gently west-dipping reflections (A, B in Fig 3) steepen pward from 2–3 s Associated structures can be followed along istinct reflections or truncations of events to near the surface ace of the ILFZ (Figs 2*b*, 3), using geometry based on migrated ections Here the reflections may correspond to zones of catalasite and mylonite up to 70 m thick[11] To the west, reflections and B merge with the base of the E package to become a iffuse zone extending to ~4 s at the western end of the comosite line The ILFZ is not represented by distinct reflections n the other lines, but is constrained by non-offset and nonuncation of reflections to dip ~15° N along line 1 and ~15° NW long line 6 The inferred extension of the ILFZ occurs at a ommon depth of ~3 8 s at the intersection of the lines and ossibly continues to ~5 s at the north end of line 1 (I, Fig 2*a*)

In the central and western parts of the survey area, the Kapuskasing zone and Wawa gneiss terrane are represented down to 6–8 s by discontinuous, rolling sub-horizontal reflections up to 5 km long (Fig 2) Again, shallow reflections can be related to rock exposures A zone of sub-horizontal reflectivity at 0 3–3 s at the northern end of line 1 (F, Fig 2*a*) is consonant with gently undulating surface structure in the overlying high-grade paragneiss, concave-down reflective zone G occurs beneath a structural dome of tonalitic gneiss Shallow reflections (J) from the thin wedge of hanging-wall rocks at the north-west end of line 6 are sub-horizontal or gently west dipping, corresponding to surface structural geometry[20]

The depth to the base of strong reflectivity varies along each line and between lines Beneath line 2-3-4 it occurs at 13 s in the east, at 6 s in the east central region, and undulates between 11 and 13 s elsewhere Reflections are abundant to at least ~13 s beneath the Abitibi belt on lines 1 and 6, but decrease markedly at 8–9 s to the north The variation in depth extent of reflectivity does not seem to correlate with crustal thickness, estimated at 14–16±0 6 s over the area of the reflection survey (Fig 1)[7,8] Although some of the change in reflectivity is attributable to varying surface conditions, it is possible that a deep-crustal deformation zone, characterized by disrupted structures and weakened reflections, characterizes the crustal bulge beneath the KU

The reflection sections have in common (1) shallow (<2 0 s) sub-horizontal or west-dipping reflections that can be projected short distances to surface structure in both the granulite-facies Kapuskasing zone and amphibolite-facies Wawa gneiss terrane, (2) images of the ILFZ, a structure dipping 16° north-north-west that can be traced to 4–5 s, (3) prominent west or north-dipping reflectors beneath the Abitibi subprovince, (4) reflections to ~13 s in the Abitibi crust by contrast with absent or less coherent reflections below 6–8 s beneath the KU, and (5) no discrete reflecting zone or marked reduction in reflectivity corresponding to the refraction-determined Moho

Based on the reflection image of the ILFZ, the KU is a much thinner feature than inferred from earlier gravity modelling[4] Traced to 4 s on line 2-3-4 and 5 s on line 1, the fault probably continues to dip northwesterly to a depth of ~20 km (6 6 s) based on refraction data from further west[7] These constraints allow us to estimate the horizontal shortening required to elevate the granulites to the surface Their depth of origin is ~20 km,

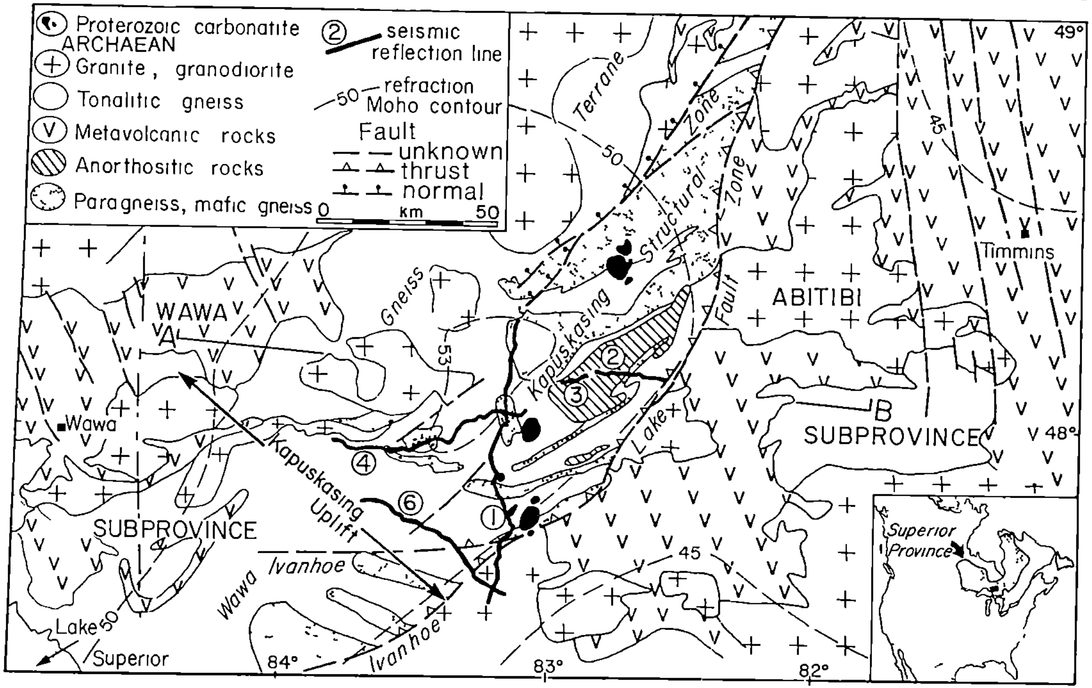


FIG 1 Simplified regional geological map with locations of seismic-reflection lines and Moho contours[8] in km

based on the palaeopressure difference of ~6 kbar between the low-grade rocks near Lake Superior and the Kapuskasing granulites, and on refraction data, linking the 6 6 km s^{-1} velocities of the Kapuskasing zone to normal depths for this velocity (20 km) near Lake Superior The shortening estimate then depends on assumptions concerning fault geometry north-west of the region surveyed If the fault dips consistently at 16° to 20-km depth, approximately 70 km of shortening is required, whereas the presence of a ramp near the north-west edge of our survey[19] would lessen this to a minimum value of 55 km

Similar magnitudes of overthrusting have been documented in many orogenic belts[21], where décollements must extend hundreds of kilometres to plate boundaries to balance shortening on a lithospheric scale Although the crustal geometry is relatively well constrained, the lateral continuation of the ILFZ i undetermined and therefore the mechanism of crustal deforma tion that caused the KU is not fully resolved Structural model that account for the shortening and associated >10-km increa in crustal thickness (Fig 4) include (1) the detachment roo at an ancient plate boundary to the north-west[19], (2) convergenc from the south-east caused underthrusting of the Abitibi rock beneath the KU (tectonic delamination[22]), and (3) brittle short ening of the upper crust was balanced by ductile flow in th lower crust (as in the Zagros collision zone[23])

Although the seismic data provide no direct evidence on th age of thrusting, the inferred 55–70 km of shortening has impli cations for the timing The arcuate 2 45-Gyr (ref 24 Matachewan–Hearst dyke swarm crosses the southern KU wit

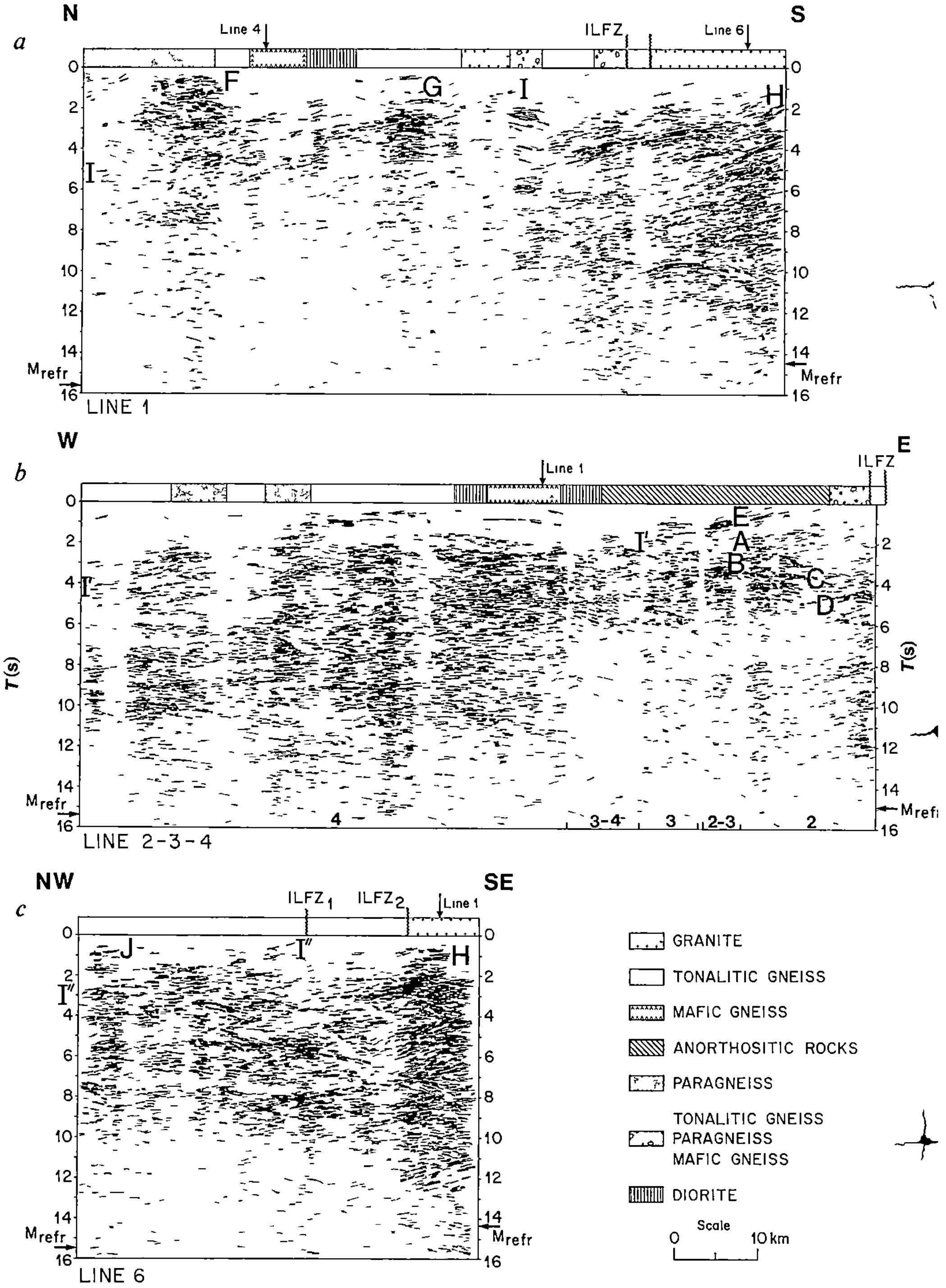
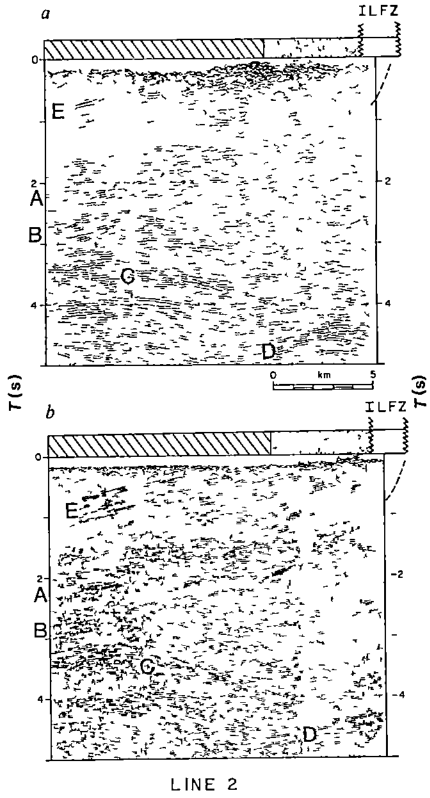


FIG 2 Line drawings of *a*, line 1 *b*, line 2-3-4 and *c*, line 6 based on unmigrated 60-fold data, an automated procedure produced similar results Parts of 2-3-4 indicated by 2-3 and 3-4 are undershoots Location of the lines is shown in Fig 1 $ILFZ_1$ and $ILFZ_2$ identify two splays of the Ivanhoe Lake fault zone A portion of the composite line 2-3-4 is shown in Fig 3 Vertical scale is travel time in seconds, approximate depth in kilometres may be obtained by multiplying travel time by three The sections are plotted at 1 1 for a velocity of 6 km s^{-1} Annotation refers to features discussed in the text



IG 3 Comparison of regional and high-resolution LITHOPROBE stacked eismic reflection data collected along the eastern segment of composite ine 2-3-4 Location of the line is shown in Fig 1 and the geological legend n Fig 2 Geology is taken from more detailed maps than shown in Fig 1 The regional data were collected with the following parameters 50-m eceiver spacing, 100-m source spacing, 240 channels, 60-fold coverage, 4 arge vibrators, 12-52 Hz sweeps, 16-s correlated record length, 4-ms ample interval The high-resolution data were collected with the following arameters 20-m receiver spacing, 20-m source spacing, 120 channels, 0-fold coverage, 2 large vibrators, 20-130 Hz sweeps, 8-s correlated record ength, 2-ms sample interval Plotting parameters as in Fig 2 Annotation efers to features discussed in the text

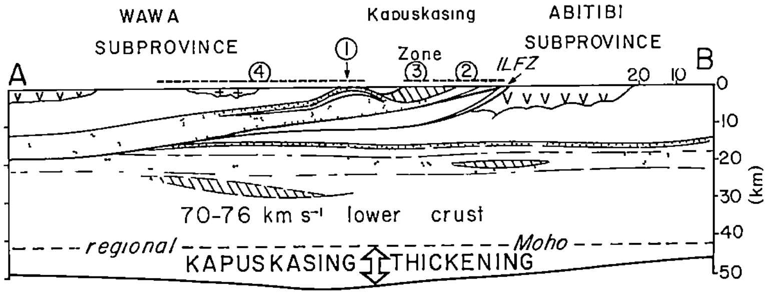


FIG 4 Summary cross-section through the Kapuskasing uplift (legend as in Fig 1), showing 70 km of horizontal shortening by thrusting and ductile thickening, respectively above and below a decollement at ~20 km depth 1 to 4 (circled) show approximate locations of the seismic reflection profiles relative to the sketch Alternative mechanisms that account for the geometry are discussed in the text

only minor offsets[25] Unless the transport direction coincidentally followed the arcuate trend of the dykes, this suggests that major movement preceded 2 45 Gyr, supporting some palaeomagnetic[26] and Ar-Ar age[27] observations and contrasting with inferences[15,28] of principal uplift at ~2 Gyr

To generally apply the model of crustal evolution developed through the three-dimensional Kapuskasing exposure, we must compare the KU seismic signature to that of other regions The general character of the Kapuskasing profiles, with abundant reflections in the upper 8 s, diminishing downwards to ~13 s, some distance above the refraction-determined Moho, is similar to other Precambrian areas[29] but differs from the pattern observed in some younger terranes of transparent upper and reflective lower crust[30] Several explanations for these crustal-scale differences are possible (1) reflectivity is enhanced by some parameter that changes with time (slowly released metamorphic fluids, for example), (2) the Kapuskasing area has a deep, nonreflective component not found in younger crusts (such as a massive underplate), (3) information from young crusts is biased towards extended terranes that have a characteristic structure, or (4) there are fundamental differences in the structure and evolution of Archaean and younger crusts Nevertheless, for the kilometre-scale, laminated, deep-crustal reflections that make up the regional patterns, the reflective, sub-horizontally-layered, high-grade rock sequences at Kapuskasing, with velocities in the 6 6 km s^{-1} range, provide a viable analogue Arc-building processes involving injection of sub-horizontal sheets of mafic and felsic magma, with attendant differentiation, metamorphism and assimilation, may therefore have been widespread during construction of the foundations of the continents □

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1 Oliver J E Brown L D & Cook F A *J geophys Res* **88**, 3329-3347 (1983)
2 Fountain D M & Salisbury M H *Earth planet Sci Lett* **5**, 263-277 (1981)
3 Bayer R *et al* *C r hebd Séanc Acad Sci Paris* Sér II **305**, 1461-1470 (1987)
4 Percival, J A & Card K D *Geology* **11**, 323-326 (1983)
5 Percival J A & Card K D in *Evolution of Archean Supracrustal Sequences, Geol Ass Can Spec Pap 28* 179-192 (1985)
6 Percival J A *Am Miner* **68**, 667-686 (1983)
7 Boland A *et al* *Nature* **335**, 711-713 (1988)
8 Boland A & Ellis R M *J geophys Res* **94**, 7189-7204 (1989)
9 Percival J A & McGrath P H *Tectonics* **5**, 553-572 (1986)
10 Cook F A *Geology* **13**, 368-371 (1985)
11 Bursnall J T in *Geol Surv Pap Can* **89-1C**, 405-411 (1989)
12 Studemeister P A *Can J Earth Sci* **20**, 1409-1420 (1983)
13 Percival J A *Am geophys Un Geodyn Ser* **14**, 135-141 (1986)
14 Percival, J A & Fountain D M in *Fluid Movements-Element Transport and the Composition of the Deep Crust* (ed Bridgwater D) 51-60 (Kluwer Dordrecht 1989)
15 Percival J A Parrish R R Krogh T E & Peterman Z E in *Proc Kapuskasing Structural Zone Transect Workshop* (ed West G F) 43-47 (University of Toronto 1988)
16 Green A G *et al* *Tectonophysics* (in the press)
17 Fountain D M, Salisbury M H & Percival J A *J geophys Res* (in the press)
18 Percival, J A *Geosci Can* **15**, 151-153 (1988)
19 Geis W T *et al* *Geology* (in the press)
20 Moser D E in *Geol Surv Pap Can* **89-1C**, 215-224 (1989)
21 Cook F A *et al* *Geol Soc Am Bull* **92**, 738-748 (1981)
22 Meissner R *Terra Rev* **1**, 17-28 (1989)
23 Snider D B & Barazangi M *Tectonics* **5**, 361-373 (1986)
24 Heaman L D *Geol Ass Can Abstr* **13**, A53 (1988)
25 West G F *Geol Ass Can Abstr* **14**, A38 (1989)
26 Symons, D T A Lewchuk M E & Vandall T A in *Proc Kapuskasing Structural Zone Transect Workshop* (ed West G F) 205-210 (Univ of Toronto 1988)

27 Queen M, Hanes J A & Archibald D A *Geol Ass Can Abstr* **14**, A123 (1989)
28 Costanzo Alvarez V & Dunlop D J *J geophys Res* **93**, 9126-9136 (1988)
29 Gibbs A K *Am Geophys Un Geodyn Ser* **14**, 95-106 (1986)
30 Matthews, D H & Cheadle M J *Am Geophys Un Geodyn Ser* **13**, 5-19 (1986)

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The hafnium paradox and the role of garnet in the source of mid-ocean-ridge basalts

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MID-OCEAN-RIDGE basalts (MORBs) are thought to result from melting in the mantle at depths of less than 60 km, in the spinel stability field[1–3]. MORBs have $^{176}Hf/^{177}Hf$ ratios indicating derivation from a mantle reservoir with a long-term Lu/Hf ratio greater than that of C1 chondrite meteorites, yet the measured Lu/Hf ratios in MORB are lower than in C1 chondrites: this is the 'hafnium paradox'[4]. Here we show that the Lu–Hf and Sm–Nd systematics of MORBs require garnet to be a residual phase in MORB melt genesis. This places the onset of melting beneath a mid-ocean ridge at depths greater than 80 km. A sequential melting model, in which melting starts in the garnet stability field and then continues at shallower levels, best explains the combined Nd and Hf isotope systematics, and is compatible with our present geophysical and geochemical knowledge of mid-ocean-ridge magmatism.

Patchett and co-workers[5–7], using hafnium isotopes to constrain geological processes, concluded that, in general, Hf isotopes in oceanic volcanics (MORB and OIB) are well correlated with Nd isotopes[5]. The Lu-Hf isotope system has therefore received only very limited attention in mantle studies. The good correlation between the Nd and Hf isotope systematics is believed to reflect similar behaviour of the parent/daughter ratios, Sm/Nd and Lu/Hf respectively, during mantle differentiation processes. We can use this similarity in behaviour and any deviation therefrom to constrain the MORB melting process.

MORBs are generally thought to form by decompression melting of a spinel lherzolite, followed by fractionation in a magma chamber before eruption. This model implies that the mantle residual to MORB genesis consists of olivine, orthopyroxene and probably some chromium diopside[8]. Estimates for the degree of melting of MORBs ranges from 1–25%[8–10]. Most MORBs are believed to be generated at pressures of at least 8–10 kbar, as the melt is not in equilibrium with orthopyroxene[3,11] below this pressure. In the anhydrous melting of spinel peridotite, the partition coefficients increase in the order Nd, Hf, Sm, Lu[12,13]. Knowledge of the solid/melt distribution coefficients allows coupling of the isotopes, that is parent/daughter ratios in the mantle source can be related to their parent/daughter ratios in the melt. The coupling of the Nd and Hf systematics and the information they contain concerning the parent/daughter ratios of the source and melt have not been modelled before. MORBs form a fairly smooth trace-element pattern on incompatibility (spider) diagrams[12], indicating possible derivation from an anhydrous spinel lherzolite. Sm/Nd, Sm/Hf and Lu/Hf ratios in MORBs are not sensitive to shallow-level crystal fractionation effects, so they will reflect the original source ratios as modified only by the melting process. The $^{176}Hf/^{177}Hf$ and $^{143}Nd/^{144}Nd$ ratios yield information on the long-term Lu/Hf and Sm/Nd ratios in the source.

Figure 1 shows the Lu/Hf and Sm/Nd ratios in MORBs an hypothetical isochrons for a MORB mantle source. All MORB values lie on the left of the 1–3 Gyr mantle isochrons, that is, the Lu/Hf and Sm/Nd ratios in MORB are too low for the observed $^{176}Hf/^{177}Hf$ and $^{143}Nd/^{144}Nd$. This indicates that the process generating MORBs has produced significant fractionation (decrease) of the Lu/Hf and Sm/Nd ratios. This observation is the Hf paradox, in that the Lu/Hf in MORB is lower than C1 chondrite, whereas $^{176}Hf/^{177}Hf$ indicates a long-term Lu/Hf higher than C1[4]. As for Sm/Nd, the Lu/Hf ratio in C1 chondrite is also the best estimate for the bulk-Earth (BE) Lu/Hf ratio[14].

To quantify these parent–daughter fractionations, we chose a 2-Gyr model age for the MORB source[14,15], a choice of 1.0 or 3.0 Gyr would not alter the arguments below. The difference between the mantle isochron value and the measured MORB value can be expressed as $\delta_{Sm/Nd} = ((Sm/Nd)_{2Gyr} - (Sm/Nd)_m)/(Sm/Nd)_{2Gyr})$, where $(Sm/Nd)_{2Gyr}$ is the calculated Sm/Nd based on the measured present-day $^{143}Nd/^{144}Nd$ of a basalt and $(Sm/Nd)_m$ is the measured Sm/Nd in this basalt. $\delta_{Lu/Hf}$ is defined in the same way, and $\delta_{Lu/Hf}$ and $\delta_{Sm/Nd}$ for MORBs and OIBs are plotted in Fig 2. A δ-value of zero means that the sample falls on a 2-Gyr isochron through BE (bulk Earth). The $\delta_{Lu/Hf}$ of MORB is by far larger than zero, which is the hafnium paradox. Some recent process must be responsible for these large δ-values.

The high $\delta_{Sm/Nd}$ in OIBs (that is, that the Sm/Nd ratios do not support the measured $^{143}Nd/^{144}Nd$) was recognized early on[16], and this phenomenon was shown to be more pronounced in the Lu-Hf system, extending to MORBs as well as OIBs[5]. The high $\delta_{Sm/Nd}$ in OIBs is thought to be caused by either recent metasomatism or by extremely small amounts of melting of a garnet lherzolite[17]. For MORBs, metasomatism is a viable, though *ad hoc*, explanation for the low Sm/Nd and Lu/Hf in the basalts, we will consider the more likely case that melting has decreased Lu/Hf and Sm/Nd in MORBs, and has led to the observed $\delta_{Lu/Hf}$ and $\delta_{Sm/Nd}$.

We used the melting equation for equilibrium non-modal partial melting to calculate the trace-element ratios in the melt. We chose the proportions in which the phases enter the melt so that the melt was basaltic in composition. Our first conclusion is that simple one-stage melting models with anhydrous spinel lherzolite as a source material cannot account for the observed $\delta_{Lu/Hf}$ in MORBs, unless extremely small amounts of melt are extracted (<0.1%). The combined $\delta_{Sm/Nd}$ and $\delta_{Lu/Hf}$ systematics cannot be satisfied with any degree of melting in the spinel stability field, using either batch equilibrium melting or any of the more complicated melting models[1,18–22]. The Sm-Nd and Lu-Hf isotope systematics cannot be simultaneously satisfied even by using an extreme set of partition coefficients for clinopyroxene (increasing D_{Lu} so that $D_{Lu}/D_{Hf} > 3$, well above the experimental values).

A phase with D_{Lu}/D_{Hf} larger than clinopyroxene but with 'comparable' Ds for Sm and Nd is required. Garnet is such a phase, the relevant partition-coefficient ratios are $D_{Lu}/D_{Hf} = 23.9$ and $D_{Sm}/D_{Nd} = 4.3$ (for clinopyroxene $D_{Lu}/D_{Hf} = 1.55$ and $D_{Sm}/D_{Nd} = 1.60$). The garnet-melt distribution coefficients are from ref 23, and our unpublished data on garnet-diopside partitioning in garnet lherzolite xenoliths, equilibrated at 19–27 kbars, from Pali-Aike, Chile[24]. Garnet has an important role in OIB genesis, it has been proposed as a residual phase in MORB genesis[9,25,26] and as an important phase controlling the trace elements in alpine peridotites[27] and abyssal peridotites[26]. Amphibole is another phase that can change the K_ds appropriately. MORBs are undersaturated in H_2O, however, and thus amphibole cannot be a residual phase during the majority of the melting in MORB genesis.

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In our melting model, which satisfies the Sm/Nd and Lu/Hf systematics, melting starts in the garnet stability field. In attempting to make the model as realistic as possible, we took the following observations into account. Recent calculations on melt extraction indicate that small amounts of melt (0.1–1%) are able to separate from the solid and ascend towards the surface[1]. We modelled this sequentially such that when a critical degree of melting (0.1–1%) is reached, this melt escapes and is accumulated at the top. The next batch is generated at shallower depth, with slightly different mineralogy (and mineral chemistry) and from a source already depleted by the previous melt extraction. Although this process is perhaps continuous, we treat it as a sequential batch process for numerical simplicity. Extracting melt only above a certain threshold, as in the critical melting model[21], would not substantially change the results.

The importance of the shape of the melting region has been stressed[18]. As the mantle material rises, the material at the ridge axis will end up higher than its neighbouring material that is somewhat off-axis[28]. Furthermore, the isotherm at the bottom of the plate is undisturbed and a ridge is not dynamically compensated[1,29]. Consequently, material directly under the ridge and material slightly off-axis will start melting at the same depth. The on-axis peridotite will rise to shallower depths and thus melt to a larger degree than the off-axis peridotite, which stops rising and 'turns the corner' at greater depth. In our model the melt generated off-axis is funnelled and collected at the centre. We assume that the decrease in degree of melting away from the ridge is linear[28,30] (see inset in Fig. 2 for further details).

Figure 2 illustrates the effect of garnet on the Sm/Nd and Lu/Hf ratios using the above equilibrium sequential batch melting model. Garnet is residual in the deeper part of the melting column. There are two useful ways (both shown in Fig. 2) to express the amount of melting in the garnet stability field: (1) the fraction of melt, of the total, that was generated in the garnet field, (2) the fraction of the on-axis melting column that lies in the garnet field. The degree of melting as seen at the surface is the average degree of melting and, because the shape of our melting region is linear, the average degree of melting is half the amount of on-axis melting. The middle of the MORB field in Fig. 2 can be reached with 10–15% melting on-axis, with one-fifth to one-third of the on-axis melting occurring in the garnet stability field. The low $\delta_{\mathrm{Sm/Nd}}$, high $\delta_{\mathrm{Lu/Hf}}$ MORBs can be generated by 15% of melting on-axis with one-third of the on-axis melt generated in the garnet field. Simpler melting models with residual garnet are also able to generate the observed variations, but smaller degrees of melting are required. Whatever melting model is chosen, some garnet is always required. Figure 2 also illustrates, as is commonly known, that many OIBs can only be generated by small degrees of melting, mostly in the garnet stability field.

Our model in Fig. 2 uses individual melt batches of 0.5%, and a garnet/diopside ratio of unity. If 0.1% of melt is allowed to escape, then slightly less on-axis melting is needed, 3–14%, to obtain the full range of $\delta_{\mathrm{Lu/Hf}}$ and $\delta_{\mathrm{Sm/Nd}}$ in MORBs. A maximum amount of on-axis melt (between 7–20%) can be obtained by extracting individual batches of 1%. The lower degrees of total melting needed with small (0.1%) individual batches is a consequence of the efficiency of this process, which effectively removes all the trace element from the residue.

The dynamic-melting model proposed by Langmuir *et al.*[20] (or the model proposed by Johnson *et al.*[26]) is also capable of explaining the $\delta_{\mathrm{Sm/Nd}}$ and $\delta_{\mathrm{Lu/Hf}}$, however smaller degrees of melting are necessary. The models proposed by McKenzie and Bickle[1], or Prinzhofer and Allègre[19] cannot generate the observed $\delta_{\mathrm{Sm/Nd}}$ and $\delta_{\mathrm{Lu/Hf}}$. The correlation between $\delta_{\mathrm{Lu/Hf}}$ and other indicators of degree of melting, such as $\mathrm{Na}_{8.0}$ (ref. 10), confirms the idea that $\delta_{\mathrm{Lu/Hf}}$ can be used as an indicator for the degree of melting. Furthermore, our estimates of the degree of melting, 5–17%, agrees with the estimates of Klein and Langmuir[10].

The onset of melting in the garnet stability field places the geotherm under the ridge on the solidus at 1,450 ± 50 °C at 80–90 km (refs 17, 31). This estimate is within the uncertainty of the Parsons and Sclater[32] estimate of 1,350 ± 275 °C at 125 km for the base of the lithosphere, but their estimate seems to be somewhat low. It is difficult to calculate whether the total amount of melt produced is realistic because the behaviour of the solidus as a function of melt depletion of the peridotite is not known. Experiments on peridotites[33] indicate an increase in the solidus by ≈50 °C for 20% batch melting of a fertile peridotite, this is a minimum estimate for a sequentially melted source. Assuming the increase in the solidus is 75 °C and using heat-of-fusion data from ref. 34, then the magma reaches 1,300–1,320 °C at 20-km depth with 15% of adiabatic batch melting. Assuming the onset of melting at 90-km depth, then the relatively large degree of melting in the garnet stability field can be explained by the effect of increased amounts of melt generated at a cusp of the solidus[35] related to the garnet–spinel transition. Although more knowledge of the solidus of depleted peridotite is necessary to test our model fully, our conclusions are consistent with the limited information derived from experimental data.

The $\delta_{\mathrm{Lu/Hf}}$ and $\delta_{\mathrm{Sm/Nd}}$ calculated above assumed a depletion age of 2 Gyr for the MORB mantle. However, an average residence time of 600 Myr for some trace elements in the MORB

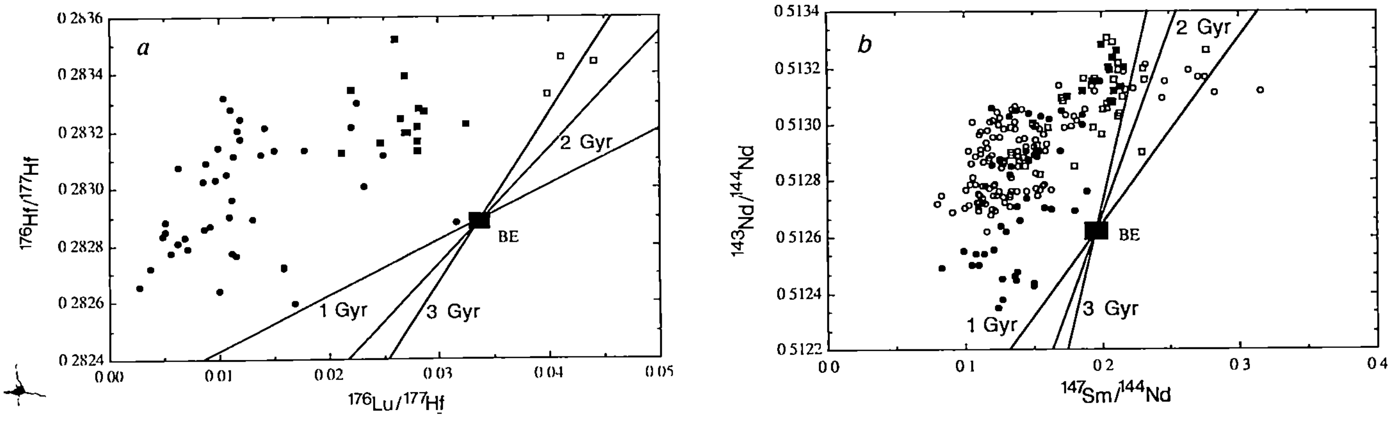

FIG. 1 *a*, $^{176}\mathrm{Hf}/^{177}\mathrm{Hf}$ plotted against $^{176}\mathrm{Lu}/^{177}\mathrm{Hf}$ and *b*, $^{143}\mathrm{Nd}/^{144}\mathrm{Nd}$ plotted against $^{147}\mathrm{Sm}/^{144}\mathrm{Nd}$ for oceanic volcanics. Squares are MORBs and circles are OIBs. Solid symbols are for those samples where both the Lu-Hf and Sm-Nd systematics are known. The 1-, 2- and 3-Gyr lines are isochrons through BE (bulk Earth). The geochron is a near-vertical line through BE (not shown). Samples falling to the left of the isochrons must have undergone a recent decrease in parent/daughter ratio. Data from refs 5, 6, 37 and references therein, W. M. White, unpublished data, and MIT, unpublished data. Bulk Earth Lu/Hf from ref. 38 and C1 (or BE) $^{176}\mathrm{Hf}/^{177}\mathrm{Hf}$ from ref. 6.

Any mantle model[1-4] includes significant amounts of both CaO and Al_2O_3, in addition to SiO_2, MgO and FeO. From high-pressure experiments performed on simple chemical systems, it has been suggested that, in the deep mantle, calcium is found in a $CaSiO_3$ perovskite[5,6], with aluminium in an Al-bearing perovskite[7,8]. Several experiments with more complex chemical systems have shown, however, that other aluminocalcic high-pressure phases could be formed. For instance, a CaO-rich phase and an Al_2O_3-rich phase have been observed in experiments performed at 27 GPa and 1,600 °C, the composition of the starting material being close to the olivine-subtracted peridotitic mantle composition[9,10]. Owing to the small grain size, the exact composition and the structure of these new phases are unknown.

To identify such aluminocalcic high-pressure phases, we have transformed a natural garnet in a laser-heated diamond-anvil cell. The starting material was a xenocryst, from Moses Rock (Arizona), with a composition close to $(M_{0.88}, Ca_{0.12})_3Al_2Si_3O_{12}$ where M corresponds to divalent cations Mg, Fe and Mn (see Table 1). The starting material was crushed in an agate mortar, and the powder was loaded in the hole of a nickel gasket. Samples were then brought to ~50(±5) GPa, as determined with a strain gauge[11], and heated by scanning the beam of a Nd YAG laser across the sample. After heating, the quenched products were ion-beam thinned and observed with a Jeol 2000 EX electron microscope equipped with a Tracor TN 5400 FX analytical attachment. The transformed materials were identified by electron diffraction and/or microanalysis. All experimental procedures, and especially the precision of chemical analysis, are described elsewhere[11,12].

In all samples, observations by transmission electron microscope (TEM) show the coexistence of two phases. The first is perfectly stable under the electron beam, and it is found in contact with another phase that amorphizes quickly under the electron beam (Fig. 1*a*). The electron diffraction patterns taken on this latter phase before amorphization are compatible with the structure of orthorombic perovskite[13], but those obtained on the stable phase are new: they do not correspond to a classical high-pressure structure such as perovskite. The composition of the two phases, given by *in situ* X-ray microanalysis, are close to $(M_{0.78}, Ca_{0.03}, Al_{0.19})(Si_{0.81}, Al_{0.19})O_3$ for the perovskite phase and $(M_{0.5}, Ca_{0.5})Al_2Si_2O_8$ for the other phase. The composition of the starting material and those of the final products (Table 1), indicate that the decomposition of garnet must also lead to the formation of a magnesiowüstite, according to the reaction:

$$(M_{0.88}, Ca_{0.12})_3Al_2Si_3O_{12} \rightarrow (4-3x)\cdot(M_{0.78}, Ca_{0.03}, Al_{0.19})(Si_{0.81}, Al_{0.19})O_3 + x\cdot(M_{0.5}, Ca_{0.5})Al_2Si_2O_8 + x\cdot MO$$

where x is a factor depending of the initial content of calcium in the starting material. In our experiments $(Ca/Ca+M=0.12)$, $x=0.557$.

No magnesiowüstite was observed in the samples, but this could be due to the small amount of this phase formed during the decomposition of garnet into post-garnet phases. Indeed, from the molar volume of each component, ~6% of magnesiowüstite should be formed in the previous reaction, for 56% of perovskite and 38% of the new phase. The relative proportions of perovskite and new phase observed in the transformation products of garnet are in good agreement with these values.

No $CaSiO_3$ perovskite was observed in the transformation products of garnet. We conclude that this phase cannot be formed at high pressure (at least at 50 GPa), when the three types of cations—calcium, aluminium and magnesium (or iron)—exist simultaneously in the starting material.

To see if a change in the chemical composition of the starting material leads to the same aluminocalcic phase, and to determine

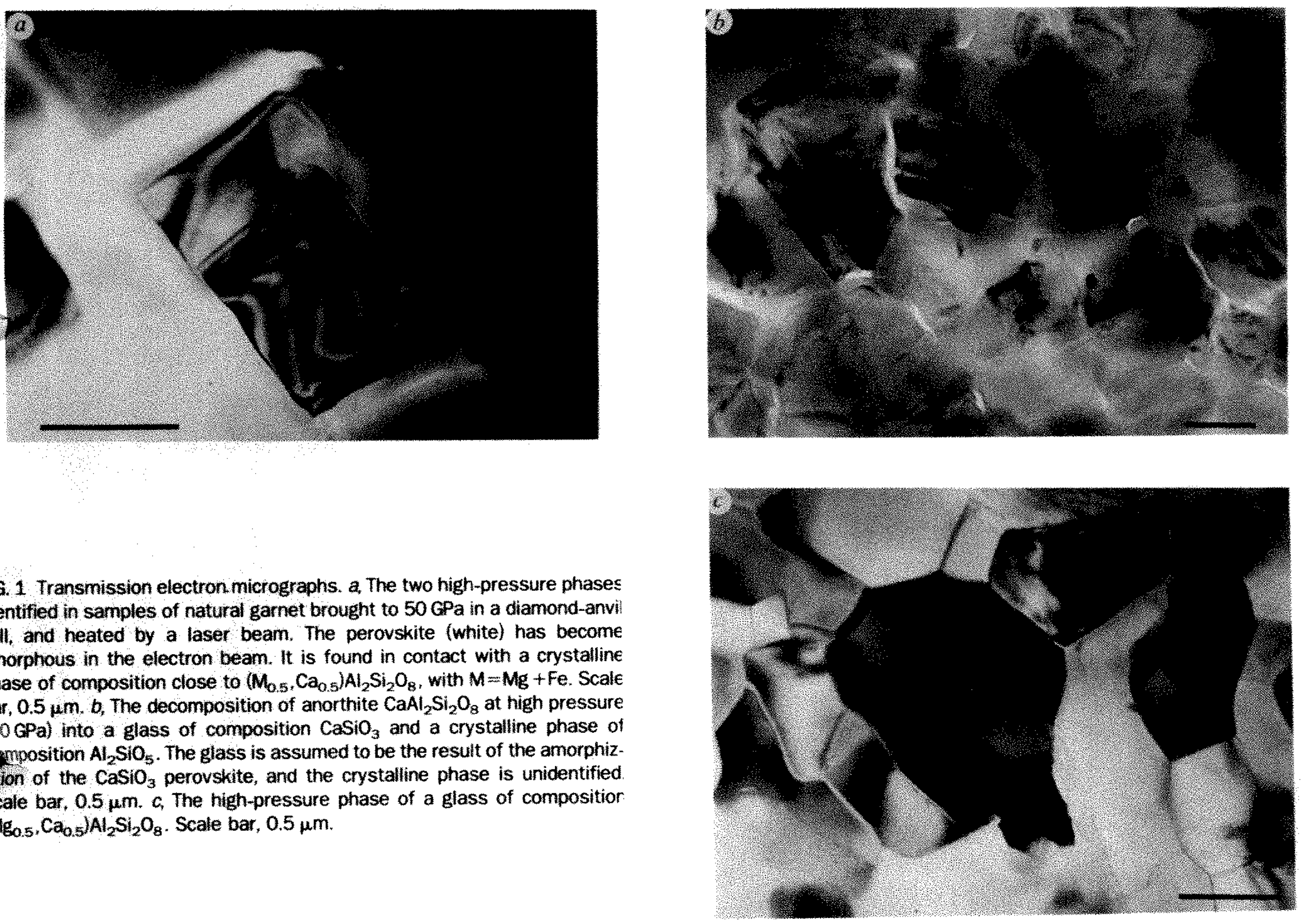

FIG. 1 Transmission electron micrographs. *a*, The two high-pressure phases identified in samples of natural garnet brought to 50 GPa in a diamond-anvil cell, and heated by a laser beam. The perovskite (white) has become amorphous in the electron beam. It is found in contact with a crystalline phase of composition close to $(M_{0.5}, Ca_{0.5})Al_2Si_2O_8$, with M = Mg + Fe. Scale bar, 0.5 μm. *b*, The decomposition of anorthite $CaAl_2Si_2O_8$ at high pressure (50 GPa) into a glass of composition $CaSiO_3$ and a crystalline phase of composition Al_2SiO_5. The glass is assumed to be the result of the amorphization of the $CaSiO_3$ perovskite, and the crystalline phase is unidentified. Scale bar, 0.5 μm. *c*, The high-pressure phase of a glass of composition $(Mg_{0.5}, Ca_{0.5})Al_2Si_2O_8$. Scale bar, 0.5 μm.

the cell parameters, we performed additional runs with two glasses as starting materials. The first glass had the composition of anorthite [$CaAl_2Si_2O_8$] and the second glass, the same composition with half atoms of magnesium in substitution of atoms of calcium [$(Ca_{0.5},Mg_{0.5})Al_2Si_2O_8$]; the latter composition is very close to that described in the previous section. The finely powdered glasses, mixed with a trace amount of platinum to absorb the laser radiation, were compressed and heated in the diamond-anvil cell at the same (P, T) conditions than those previously described. The TEM observations of the transformation products of two glasses lead to the following comments: (1) At high pressure, $CaAl_2Si_2O_8$ decomposes in two phases, a glass of composition $CaSiO_3$, in contact with another crystalline phase of composition Al_2SiO_5 (Fig. 1*b*). The amorphous $CaSiO_3$ phase is assumed to be the result of the amorphization of $CaSiO_3$ perovskite at room pressure, as generally observed[14], and the crystalline Al_2SiO_5 phase is unidentified, but the electron diffraction patterns disagree with the kyanite structure; (2) The experiments done with the second glass confirm the existence of a crystalline phase of $(Ca_{0.5},Mg_{0.5})Al_2Si_2O_8$ composition, at 50 GPa (Fig. 1*c*). These experiments confirm the stability of the $CaSiO_3$ perovskite at high pressure when two types of cations (Ca and Al) exist in the starting material, but also show that another high-pressure phase is preferred when the three cations (Ca, Al and Mg or Fe) are simultaneously present in the sample.

The cell parameters of the new phase were identified from powder X-ray diffraction patterns obtained with a 180-mm Debye–Scherrer camera. X-ray diffraction data for a glass sample of $(Ca_{0.5},Mg_{0.5})Al_2Si_2O_8$, quenched from ~50 GPa, are listed in Table 2. The unit-cell dimensions were determined to be $a = 9.384$, $b = 8.148$ and $c = 9.258$ Å, with $\beta = 90.474°$. The molar volume and the density of the new phase calculated for $Z = 6$, are $71.06 \pm 0.04\ cm^3\ mol^{-1}$ and $3.804 \pm 0.002\ g\ cm^{-3}$ respectively, at room temperature and pressure. This phase is very similar to the hollandite structure. First, the molar volume and the density are similar to those of $NaAlSi_3O_8$ hollandite[1] ($V = 71.0\ cm^3\ mol^{-1}$, $\rho = 3.69\ g\ cm^{-3}$) and $KAlSi_3O_8$ hollandite[16] ($V = 72.6\ cm^3\ mol^{-1}$, $\rho = 3.84\ g\ cm^{-3}$). Second, the composition of the (Ca,Mg) phase conforms to the general formula $[A_x][B_y,C_{4-y}]O_8$ of the hollandite structure family where the A cations may be monovalent or divalent, where the smaller cations making up the octahedral $[B,C]_4O_8$ framework include tri- and tetravalent species[17]. Third, the lattice parameters are very close to those of the distorded hollandite structure. The ideal hollandite unit cell is tetragonal, with a value of the '*c*' axis ~2.8 Å. This value represents the edge-to-edge dimension of a $[B,C]O_6$ octahedra. Yet, depending on the chemistry of the sample, the *c* axis can be a multiple of the ideal value. This is the case in many barium titanate hollandites where a deficiency in stoichiometry leads to a multiplicity of the *c* axis[18]. On the other hand, many hollandites show a small distortion that lowers the symmetry from the tetragonal to the monoclinic structure, with a value of $\beta \sim 90°$ (refs 19, 20). In our experiments the aluminocalcic phase seems to adopt such a distorted hollandite structure, that is a monoclinic structure ($\beta = 90.474°$) with a multiplicity of the *b* axis (equivalent to the *c* axis of the ideal structure) close to three. But two points disagree with this interpretation. First, the multiplicity of the *b* axis is not easily explained because the (Ca,Mg) phase seems to be stoichiometric. Second, the intensities of the diffraction lines (Table 2) are not in good agreement with those of the ideal hollandite structure. Further work is needed to resolve the issue.

The density of the $(Ca_{0.5},Mg_{0.5})Al_2Si_2O_8$ phase seems to be low at room pressure ($\rho = 3.804\ g\ cm^{-3}$) with respect to the densities of other high-pressure phases such as $MgSiO_3$

TABLE 1 Chemical composition (in atom %) of the starting material (garnet) and of the three post-garnet components

Element	Garnet*	Perovskite†	(Ca,Mg)-phase†	Magnesiowüstite‡
Si	15.00	16.20	15.18	00.00
Ti	00.03	00.02	00.02	00.00
Ca	01.80	00.57	04.05	00.00
Mg	10.05	13.35	03.24	20.64
Fe	03.04	02.12	00.82	28.24
Mn	00.11	00.08	00.01	01.12
Al	09.66	07.35	15.05	00.00
Cr	00.26	00.25	00.25	00.00
O§	60.00	59.96	61.47	50.00

* Analyses from a Camebax (Camparis, Université Paris 6) electron microprobe.

† Analyses from the Tracor TN5400 energy-dispersive X-ray analyser.

‡ Estimated composition of magnesiowüstite (see text).

§ Oxygen given by stoichiometry.

TABLE 2 X-ray powder diffraction data for $(Ca_{0.5},Mg_{0.5})Al_2Si_2O_8$ phase

h	*k*	*l* monoclinic	d_{obs}	d_{calc}	I/I_0
1	2	0	3.738	3.736	8
1	−2	−1	3.467	3.469	10
3	0	0	3.130	3.128	17
1	0	−3	2.937	2.939	26
0	3	0	2.702*	2.716	29
0	3	−1	2.607	2.606	63
2	1	−3	2.468	2.467	47
0	3	−2	2.341	2.343	7
1	0	4	2.243	2.243	94
3	0	3	2.189	2.188	33
0	3	3	2.040	2.039	100
1	2	−4	1.972	1.971	25
3	2	−3	1.941	1.940	43
4	2	−2	1.866	1.866	9
2	0	−5	1.728	1.727	12
1	2	5	1.658	1.657	5
5	0	3	1.599	1.598	23
6	0	0	1.563	1.564	57
4	4	0	1.537	1.538	40
0	1	6	1.516	1.516	6
2	0	−6	1.469	1.469	<5
2	1	6	1.439	1.439	<5
6	0	−3	1.400	1.400	<5
4	−4	3	1.373	1.374	21
1	6	0	1.345	1.344	34
3	5	3	1.307	1.307	<5
1	6	3	1.232	1.232	<5
2	6	−3	1.202	1.202	<5
4	6	0	1.175	1.175	22
1	0	8	1.147	1.147	<5
7	3	−3	1.123	1.123	6

* Reflection not used for lattice-constant refinement.

Calculated from $a = 9.384(2)$, $b = 8.148(2)$, $c = 9.258(2)$ Å and $\beta = 90.474(22)°$. $V = 707.89$ Å^3($71.06\ cm^3\ mol^{-1}$), $\rho = 3.804\ g\ cm^{-3}$.

TABLE 3 Estimates of mineralogical constitution of the lower mantle (in volumetric ratio) for different models of chemical composition

Composition model	Pyrolite*	Lherzolite†	Chondritic†	Piclogite†
Perovskite $(M_{0.97},Ca_{0.03})SiO_3$	65.0	70.5	76.6	55.2
Magnesiowüstite MO	20.0	23.0	11.6	9.2
(Ca,Mg)-phase $(M_{0.5},Ca_{0.5})Al_2Si_2O_8$	12.7	6.0	10.5	25.6
Perovskite $CaSiO_3$	2.3	0.5	1.3	10.0

Abundance of elements in each composition model is described elsewhere[4,27]. M = Mg + Fe.

* See ref. 27.

† See ref. 4.

perovskite[21,22]($\rho = 4.11$ g cm^{-3}), or $CaSiO_3$ perovskite[23,24]($\rho = 4.24$ g cm^{-3}). As $(Ca_{0.5}, Mg_{0.5})Al_2Si_2O_8$ forms at 50 GPa along with the magnesian perovskite, we can expect that its density is similar to that of perovskite $MgSiO_3$ (or $CaSiO_3$) at the same pressure. At 50 Gpa and room temperature, the perovskite densisities are ~4.9 g cm^{-3}. To obtain the same density at the same pressure, the $(Ca_{0.5}, Mg_{0.5})Al_2Si_2O_8$ phase should have a bulk modulus of ~120 GPa with the commonly made assumption ($K'_0 = 4$) for the first derivative of K_0 at 1 bar. We note that this value is low in comparison with those of perovskites ($K_0 = 247$ GPa in $MgSiO_3$ perovskite[22] and 268 GPa in $CaSiO_3$ perovskite[24]), but of the same order of magnitude as other compounds such as wüstite MgO (ref. 25) ($K_0 = 154$ GPa) or CaO (ref. 26) ($K_0 = 111$ GPa). Another explanation for the low value of the density of the aluminocalcic phase at room pressure could be a phase change of this compound at higher pressure—indeed, a reversible transition (similar to the B1-B2 transition occuring in the CaO compound at ~60 or 70 GPa (ref. 26) could lead the $(Ca_{0.5}, Mg_{0.5})Al_2Si_2O_8$ phase to a density similar to those of perovskites at high pressure and to a low density at room pressure.

We have started static measurements of the compression of the $(Ca_{0.5}, Mg_{0.5})Al_2Si_2O_8$ phase in a diamond-anvil cell to determine its high-pressure density and look for a possible phase transition.

These observations show that an aluminocalcic phase with a composition $[(Ca_{0.5}, Mg_{0.5})Al_2Si_2O_8]$ close to but different from that of anorthite, can accommodate the calcium at high pressure. We suggest that this phase could be the crystalline host for not only the calcium in the lower mantle, but also for the other large cations such as sodium, potassium or the radioactive elements. This phase could be an important constituent of the lower mantle. Depending on the assumed mantle composition, this phase is inferred to be the second or the third most abundant constituent of the lower mantle, with the perovskite and the magnesiowüstite (Table 3). Indeed, assuming that all the aluminium reacts with the calcium to form an aluminocalcic phase of the above composition, and that the solubility of the $CaSiO_3$ molecule in $MgSiO_3$ orthorhombic perovskite is ~3%, the proportion of the (Ca,Mg)-phase, in volumetric ratio, would vary from 6% to 25% of the lower mantle. As a result, the abundance of $CaSiO_3$ perovskite in the lower mantle would be negligible. □

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1. Ringwood, A. E. *Composition and Petrology of the Earth's mantle*, (McGraw-Hill, New York, 1975).
2. Maaloe, S. & Aoki, K. *Contr. Miner. Petrol.* **63,** 161–173 (1977).
3. Anderson, D. L. in *High Pressure Research in Geophysics* (eds Akimoto, S. & Manghnani, M. H.) 301–308 (1982).
4. Anderson, D. L. & Bass, D. J. *Nature* **320,** 321–328 (1986).
5. Xiong, D. H., Ming, L. C. & Manghnani, M. H. *Phys. Earth planet. Inter.* **43,** 244–252 (1986).
6. Tamai, H. & Yagi, T. *Phys. Earth planet. Inter.* **54,** 370–377 (1989).
7. Jeanloz, R. & Thompson, A. B. *Rev. Geophys. Space Phys.* **21,** 51–74 (1983).
8. Irifune, T. & Ringwood, A. E. *Earth planet. Sci. Lett.* **86,** 365–376 (1987).
9. Ito, E. & Takahashi, E. in *High Pressure Research in Mineral Physics* (eds Manghnani, M. H., Syono, Y., Ito, E. & Takahashi, E.) 221–229 (1987).
10. Takahashi, E. & Ito, E. in *High Pressure Research in Mineral Physics* (eds Manghnani, M. H., Syono, Y., Ito, E. & Takahashi, E.) 427–437 (1987).
11. Madon, M., Guyot, F., Peyronneau J. & Poirier, J. P. *Phys. Chem. Miner.* **16,** 320–330 (1989).
12. Guyot, F., Madon, M., Peyronneau, J. & Poirier, J. P. *Earth planet. Sci. Lett.* **90,** 52–64 (1988).
13. Yagi, T., Mao, H. K. & Bell, P. M. *Phys. Chem. Miner.* **3,** 97–110 (1978).
14. Liu, L. G. & Ringwood, A. E. *Earth planet. Sci. Lett.* **14,** 1079–1082 (1975).
15. Liu, L. G. *Earth planet. Sci. Lett.* **37,** 438–444 (1978).
16. Ringwood, A. E., Reid, A. F. & Wadsley, A. D. *Acta crystallogr.* **23,** 1093–1095 (1967).
17. Kesson, S. E. & White, T. J. *J. Solid St. Chem.* **63,** 122–125 (1986).
18. Kesson, S. E. & White, T. J. *Proc. R. Soc.* A **408,** 295–319 (1986).
19. Post, J. E., Von Dreele, R. B. & Buseck, P. R. *Acta crystallogr.* B**38,** 1056–1065 (1982).
20. Mukherjee, B. *Acta crystallogr.* **13,** 164–165 (1960).
21. Ito, E. & Matsui, Y. *Earth planet. Sci. Lett.* **38,** 443–450 (1978).
22. Kudoh, Y., Ito, E. & Takeda, H. *Phys. Chem. Miner.* **14,** 350–354 (1987).
23. Mao, H. K. *et al. J. geophys. Res.* (in the press).
24. Tarrida, M. & Richet, P. *Geophys. Res. Lett.* (in the press).
25. Richet, P., Mao, H. K. & Bell, P. M. *J. geophys. Res.* **94,** 3037–3045 (1989).
26. Richet, P., Mao, H. K. & Bell, P. M. *J. geophys. Res.* **93,** 15279–15288 (1989).
27. Jackson, I. *Earth planet. Sci. Lett.* **62,** 91–103 (1983).

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Discovery of the earliest-known tetrapod stapes

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THE evolution of the middle ear is central to the discussion of how the first tetrapods adapted to life on land as well as their phylogeny[1–3]. Here I report the discovery of the stapes of *Acanthostega gunnari*, from the Upper Devonian of east Greenland. This is the earliest tetrapod stapes so far described, and it throws new light on both these aspects of early tetrapod biology. It has been assumed that the common inheritance of all early tetrapods was a light, rod-like stapes associated with a temporal notch in the otic region that was thought to have supported a tympanum, or eardrum. The stapes would have conducted vibrations from the tympanum to the otic capsule. By contrast, the stapes of *Acanthostega* was stout with a broad distal ramus associated with the temporal notch. I suggest that the temporal notch of *Acanthostega* and other early tetrapods supported a spiracular opening rather than a tympanum, and that the stapes controlled palatal and spiracular movements in ventilation.

Of six *Acanthostega* stapes collected by a Cambridge-Copenhagen expedition to east Greenland (Figs. 1, 2; ref. 4), four are preserved approximately in life-position. This indicates that the stapes was firmly held in place in life, despite the incomplete ossification of the otic region of the braincase into which the footplate of the stapes was presumably inserted. The footplate, the medial surface of the stapedial head, is large. Only one head

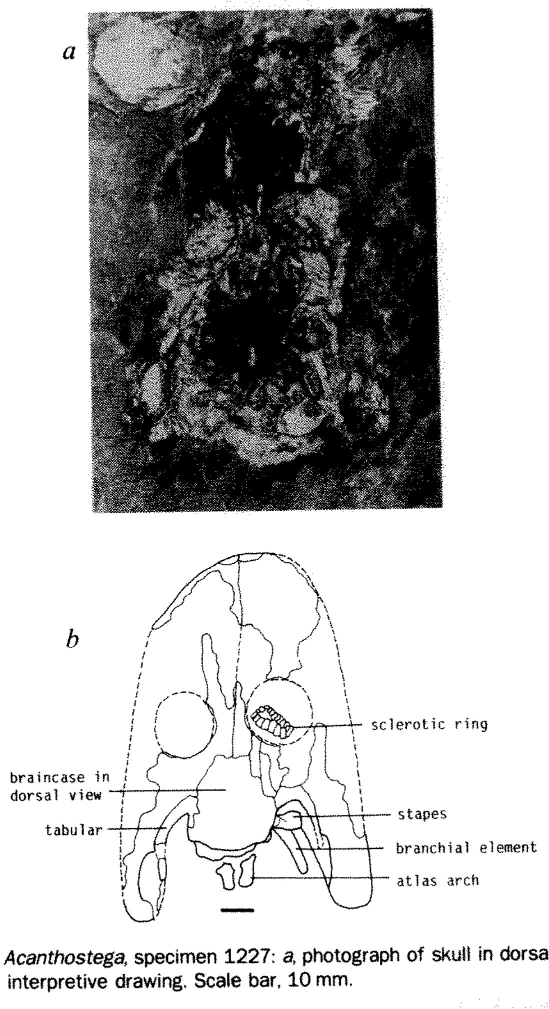

FIG. 1 *Acanthostega*, specimen 1227: *a*, photograph of skull in dorsal view with *b*, interpretive drawing. Scale bar, 10 mm.

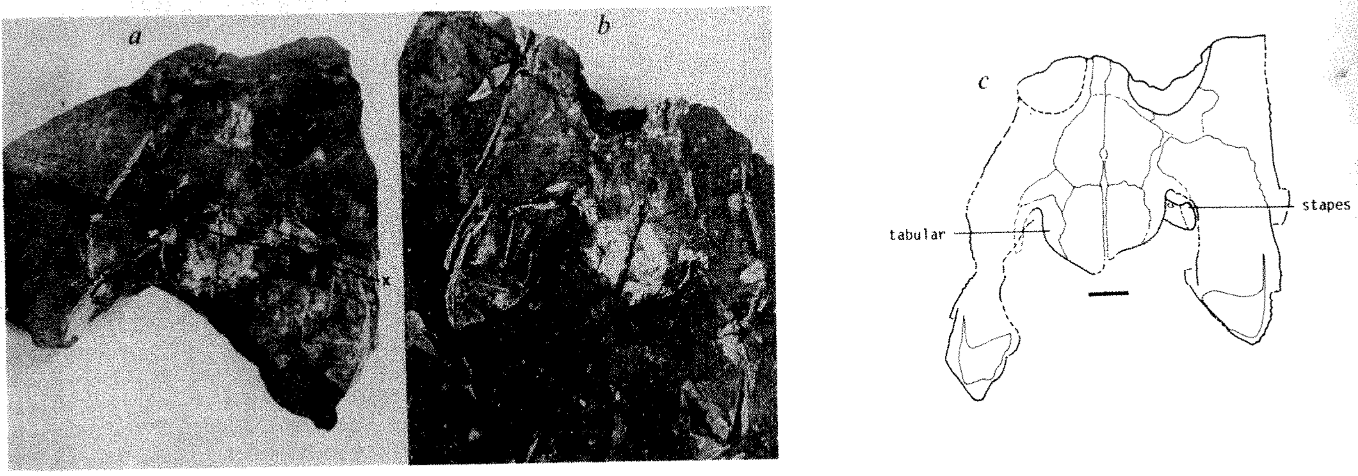

FIG. 2 *Acanthostega*, specimen 1604: *a*, partial skull in dorsal view showing proximal end of stapes; *b*, counterpart skull roof showing distal end of stapes; *c*, interpretive drawing with stapes drawn from part and counterpart of specimen. Scale bar, 10 mm.

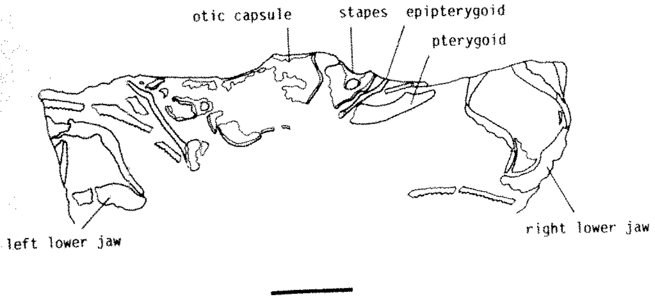

FIG. 3 *Acanthostega*, specimen 1604: drawing of section at X (see Fig. 2), showing stapes, palatal bones and lower jaws. Scale bar, 10 mm.

is apparent in vertical section, although this cannot be confirmed in the surface-prepared material, because the footplate is obscured (Figs. 3 and 4). A robust neck region separates the head from the shaft. Proximally, the head abuts the otic capsule, and the distal margin of the fan-shaped ramus, which has cartilage-finished lateral and posterior margins, extends laterally towards the well-developed temporal notch bordered by the tabular bone (Fig. 4). Temporal notches are often described as 'otic' with the presumption that each held a tympanum. Their occurrence in the earliest tetrapods has been equated with presence of a tympanum in these animals.

The massive, expanded stapes of *Acanthostega* is strikingly similar to those of two unrelated Carboniferous tetrapods, although it is much older[5,6]: *Greererpeton*, a colosteid, lacks a temporal notch[5,7], whereas *Pholiderpeton*, an embolomere, has a narrow, wedge-shaped notch (Fig. 4). The apparent absence of a tympanic ear in these Carboniferous forms could have been a convergent loss related to a secondarily aquatic lifestyle[6]. But the stapedial morphology of *Acanthostega* indicates that it too lacked a tympanic ear, though it possessed a notch. Absence of a tympanum now appears primitive for tetrapods; its presence could only reasonably be inferred if a temporal notch were associated with a relatively light, rod-like stapes. However, even in the absence of a tympanum, the stapes is likely to have had some auditory function because of the close association between the footplate and the otic capsule[8].

Carroll[7] suggested that the stapes of *Greererpeton* braced the braincase against the cheek, representing the primitive tetrapod condition in which the occiput, braincase and skull roof were not fully integrated. But the braincase and skull roof were intimately connected in both *Pholiderpeton*[9] and *Acanthostega*[10], so that a bracing function for the stapes is unlikely in these animals. I suggest instead that the stapes of primitive tetrapods was actively involved in ventilation, as was its homologue, the hyomandibula of fishes.

In many fossil fishes, including the osteolepiforms usually regarded as the closest relatives of tetrapods, the hyomandibula contributed to the suspension of the lower jaw and supported the back of the palate. By analogy with living fishes, it acted as

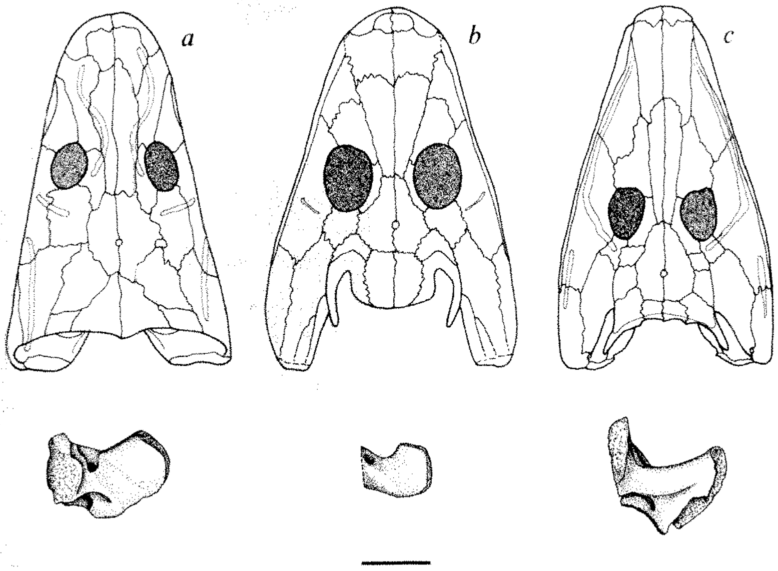

FIG. 4 Skulls and stapes of early tetrapods: *a*, *Greererpeton* (snout to quadrate length 131 mm), stapes in dorsal view below; *b*, *Acanthostega* (snout to quadrate length 115 mm), restoration of stapes from specimens 1227 and 1604 in approximately dorsal view below; *c*, *Pholiderpeton* (snout to quadrate length 320 mm), stapes in approximately dorsal view below. Scale bar (for stapes only), 10 mm.

a hinge coordinating kinetic movements of the palate and cheek used to ventilate the gills and in feeding. A similar buccal pumping mechanism is used to ventilate both lungs and gills in those fishes that have lungs[11]. These movements are controlled by muscles attached to the palate and hyomandibula, and in the modern but primitive actinopterygian *Polypterus*, these muscles also control the operation of an open spiracle[12].

The earliest tetrapods may have retained a fish-like breathing mechanism. In *Acanthostega* and other primitive tetrapods the palate and braincase were connected by a synovial joint[9,13] allowing a degree of intracranial mobility. The temporal notch in some primitive tetrapods may have housed an open spiracle homologous with the spiracular notch of the earliest bony fishes[14]. The spiracle in tetrapods could have been used for inspiration when the rest of the animal's head was submerged. The spiracular cleft and stapes could also have contributed to an underwater hearing mechanism[15]. Associated with intracranial mobility and an open spiracle, the stapes in early tetrapods might thus have retained a function more directly comparable with that of the fish hyomandibula, in that it controlled palatal and spiracular movements during breathing.

The close morphological similarities between the stapes of three otherwise distantly related tetrapods indicates that the massive stapes is a tetrapod autopomorphy, derived with respect to that of any non-tetrapod outgroup, but nonetheless primitive. Other forms, including the rod-like type found in tympanic ears, were later derivations. Alternatively, the massive stapes could be a synapomorphy uniting 'labyrinthodonts'[3], not otherwise considered to be a natural group[16]. The relationship of *Acanthostega* to any 'labyrinthodont' is not yet supported by other synapomorphies. It has recently been suggested that possession of a double-headed stapes was the primitive tetrapod condition, comparable with the double-headed hyomandibula seen in osteolepiform fishes, and the stapes of many early reptiles[17]. This view would be corroborated by finds of double-headed stapes in the earliest tetrapods, but is not supported so far by the condition actually found in those where it is known. ☐

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1. Watson, D. M. S. *Proc. zool. Soc. Lond.* B **1917,** 167–186 (1917).
2. Panchen, A. L. in *Studies in Vertebrate Evolution* (eds Joysey, K. A. & Kemp, T. S.) 65–68 (Oliver and Boyd, Edinburgh, 1972).
3. Bolt, J. R. & Lombard, R. E. *Biol. J. Linn. Soc.* **24,** 83–99 (1985).
4. Panchen, A. L. *Nature* **333,** 704 (1988).
5. Smithson, T. R. *Zool. J. Linn. Soc.* **76,** 29–90 (1982).
6. Clack, J. A. *Zool. J. Linn. Soc.* **79,** 121–148 (1983).
7. Carroll, R. L. in *The Terrestrial Environment and the Origin of Land Vertebrates* (ed. Panchen, A. L.) *Syst. Ass. Spec. Vol.* **15,** 293–317 (Academic, London, 1980).
8. Jaslow, A. P., Hetherington, T. E. & Lombard, R. E. in *The Evolution of the Amphibian Auditory System* (ed. Fritzsch, B.) 69–91 (Wiley, New York, 1988).
9. Clack, J. A. *Phil. Trans. R. Soc.* B **318,** 1–107 (1987).
10. Clack, J. A. *Palaeontology* **31,** 699–724 (1988).
11. Randall, D. J., Burggren, W. W., Farrell, A. P. & Haswell, M. S. *The Evolution of Air-breathing in Vertebrates* (Cambridge University Press, 1981).
12. Allis, E. P. *J. Anat.* **56,** 189–294 (1922).
13. Panchen, A. L. *Phil. Trans. R. Soc.* B **263,** 279–326 (1972).
14. Carroll, R. L. in Panchen A. L. *Phil. Trans. R. Soc.* B **309,** 461–568 (1985).
15. Panchen, A. L. *Phil. Trans. R. Soc.* B **309,** 461–568 (1985).
16. Smithson, T. R. *Zool. J. Linn. Soc.* **85,** 317–410 (1985).
17. Lombard, R. E. & Bolt, J. R. in *The Evolution of the Amphibian Auditory System* (ed. Fritzch, B.) 37–67 (Wiley, New York, 1988).

Ocular dominance plasticity in adult cat visual cortex after transplantation of cultured astrocytes

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DURING a critical restricted period of postnatal development, the visual cortical circuitry is susceptible to modifications that are dependent on experience[1]. If vision is restricted to only one eye during this period, the territories innervated by the deprived eye shrink considerably, whereas those innervated by the non-deprived eye expand, and the deprived eye loses the ability to influence almost all of the cells in the cortex[2,3]. Thus, changes in ocular dominance are paralleled and possibly mediated by synapse elimination and axonal sprouting. Hypotheses about the mechanisms underlying ocular-dominance plasticity assume the activation of NMDA (*N*-methyl-D-aspartate) receptors and subsequent calcium influx as a trigger of synaptic modifications[1,4,5]. In addition, plasticity relies on functional neuromodulatory afferents[6,7]. On the basis of immunocytochemical studies, it was recently proposed that the presence of immature astrocytes is a prerequisite for visual cortical plasticity, and that the end of the critical period is causally linked to the maturation of astrocytes[8,9]. Here we report, in support of this hypothesis, that resupplementation of the visual cortex of adult cats with astrocytes cultured from the visual cortex of newborn kittens reinduces ocular-dominance plasticity in adult animals.

The study described here was designed to test the hypothesis that the presence of immature astrocytes is a prerequisite for ocular dominance (OD) plasticity by transplanting astrocytes cultured from newborn kittens into the visual cortices of five adult cats (Fig. 1). These animals were at least one year old and had therefore passed the critical period for cortical plasticity (for a review see ref. 1). Controls included animals ($N = 2$) that each received a transplantation of fibroblasts (the chief contaminant of the astrocytic cultures) in one hemisphere. In addition, every animal received a transplantation of cells destroyed by repeated freezing in the hemisphere contralateral to the one transplanted with intact cells. After 4-8 weeks of monocular deprivation, which began at the time of transplantation, animals were reanaesthetized and the OD distribution assessed using standard electrophysiological techniques. To avoid a bias caused by preferential recording within certain OD columns, electrode tracks were aimed in an oblique direction in the cortex. Recording tracks were placed at the lateral coordinates where the transplants had been performed. For each cell we determined the OD (five-class scale) and the response strength (four-class scale) by presenting optimally oriented, moving light-bars with a hand lamp on a tangent screen.

The mean response strengths observed in the experimental hemispheres (transplanted with intact cells) and the control hemispheres (transplanted with cells frozen before transplantation) do not differ significantly from one another (control: 2.98 ± 0.67, $N = 321$; experimental: 3.00 ± 0.65, $N = 548$). Significant differences in the OD distributions exist between experimental and control hemispheres, however. The OD distributions of the control hemispheres that received a transplantation of cells destroyed by multiple freezing showed a contralateral bias typical for normally raised adult cats[10,11]. Most cells were driven by stimulation of either eye, and the OD distribution showed a moderate bias towards the contralateral eye (Fig. 2; far-left histogram, control). This bias occurred irrespective of whether the ipsilateral- or contralateral eye was closed during the period of monocular deprivation as a result of suturing (see Fig. 3 for data from individual animals). By contrast, the hemispheres that received intact astrocyte suspension showed OD distributions always biased towards the non deprived eye. In cases in which

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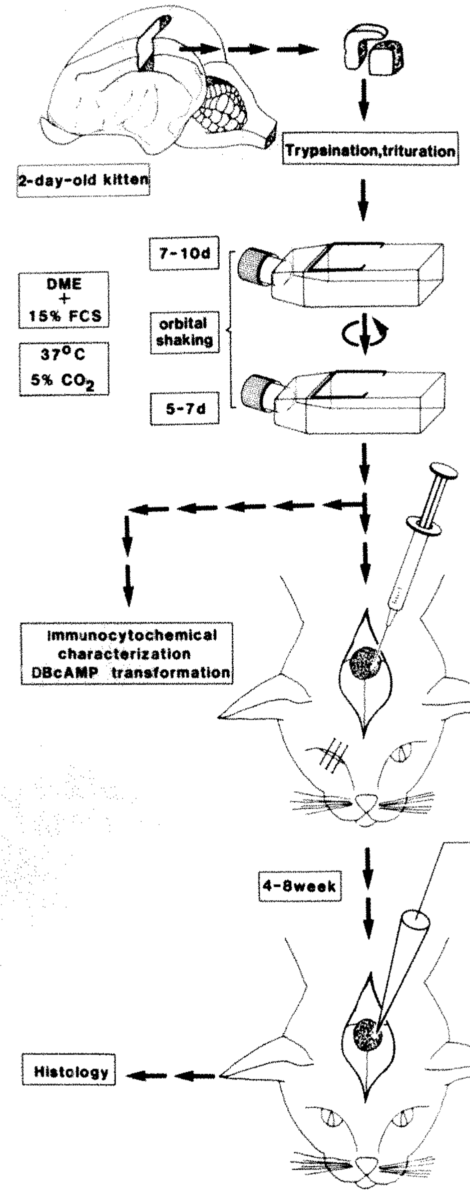


FIG. 1 Schematic representation of the experimental paradigm. Astrocytic cultures were prepared from the visual cortex of newborn kittens and injected as a cell suspension into the visual cortex of adult cats. At the same time one eye was sutured close. After a period of 4–8 weeks the OD distribution in the hemispheres was assessed by electrophysiological recording.
METHODS. Glial cultures were prepared from 2-day-old kittens. After decapitation, a block of visual cortex was removed, cleared of meningeal tissue and white matter, and minced in small pieces by use of two scalpel blades. After preincubation in Ca^{2+}- and Mg^{2+}-free phosphate buffer (CMF, 37 °C, 10 min), the tissue was transferred to a trypsin solution (1% in CMF, 37 °C, 10 min). After three washes with Dulbecco modified Eagles medium containing 10% fetal calf serum, the tissue was triturated with a fire-polished glass pipette (10 strokes) and seeded in precoated culture flasks (Primaria, 25 cm^2) at a density of 30,000 cells cm^{-2}. The culturing medium (DMEM, 10–15% FCS) was changed every second day. Contaminating oligodendrocytes were removed by orbital shaking[28] at day 7–10. Fibroblast cultures were prepared from meningeal tissue under identical culturing conditions. When cultures had again reached confluency, cells were collected either by mild trypsinization or by mechanical scraping, centrifuged and resuspended in 40 μl CMF. Part of the cultures were reseeded on glass coverslips, and the astrocytic nature of the cells was reconfirmed by immunostaining with the astroglial markers vimentin, S-100 and GFAP, in addition to testing the ability of dibutyryl cyclic AMP to transform the cells into process-bearing stellate cells. Quantification of the cultures revealed that 94% (±4%) of the cells were immunoreactive for both GFAP and vimentin and transformed into stellate cells after dibutyryl cAMP. The remaining cells were immunoreactive for vimentin only. Most of these cells had a flat morphology even after 24 h of treatment with dibutyryl cAMP and, thus, resembled fibroblasts. A few small cells containing vacuoles and resembling macrophages were encountered in astrocyte-enriched, as well as in fibroblast cultures. After collecting the cell suspension was immediately injected into the visual cortex of anaesthetized adult cats mounted in a stereotaxic frame. One hemisphere received a total of 10–20 μl (2–8 injection sites) of intact cell suspsension, and the other was injected with the same amount of cell suspension frozen and thawed three times. Transplants were placed on the top of the gyrus suprasplenius at antero-posterior coordinates −5 to +2. After cell transplantation, one eyelid was sutured closed. The animals received antibiotic treatment for three days. After a period of 4–8 weeks of monocular deprivation, the animals were reanaesthetized, and the OD distribution in the cortices was assessed by standard electrophysiological techniques, using laquer-insulated tungsten electrodes. After termination of the experiments, animals were perfused with fixative, and frozen sections were prepared for reconstruction of penetration tracks, using small electrolytic lesions as references.

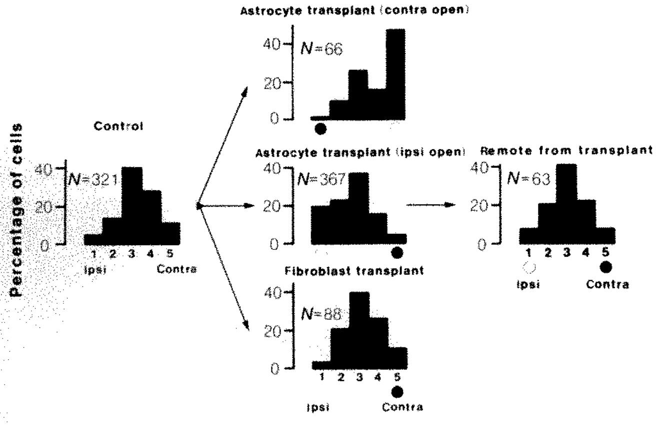


FIG. 2 OD histograms composed from data from seven cats. Monocular cells responding exclusively to stimulation of one eye are categorized in class 1 (driven by ipsilateral eye) or class 5 (driven by contralateral eye). Classes 2 and 4 represent binocular cells in which one eye was dominant. Class 3 represents cells responding equally well to stimulation of either eye. Histograms from the control hemispheres of all seven cats (far-left histogram, control) reveal a moderate bias towards the contralateral eye. The middle OD histograms were obtained from transplanted hemispheres. Combining astrocyte transplantation with deprivation of the ipsilateral eye resulted in an increased occurrence of cells driven exclusively by the experienced contralateral eye (class 5, top histogram, contra open). The central OD (ipsi open) was obtained from the astrocyte-transplanted hemisphere of four cats in which the contralateral eye was sutured closed. Note the bias towards the nondeprived ipsilateral eye. This effect was not present when recordings were obtained from locations remote from the transplantation site (far-right histogram, composite data from two animals). The bottom histogram shows data from two cats that received a transplantation of fibroblasts instead of astrocytes; the OD distribution does not differ from that obtained from control hemispheres. ○, Eye open; ●, eye closed.

the contralateral eye was closed, there is an ipsilateral dominance, whereas in one animal in which the ipsilateral eye was deprived, an overproportional number of cells were responsive only to contralateral stimulation (OD class 5; see Figs 2 and 3). The differences between the OD distributions of the control hemispheres and the astrocyte-receiving hemispheres are highly significant (χ^2 test, 4 degrees of freedom) in each cat that received an astrocyte transplantation (see Fig. 3 legend).

In addition to the control in which destroyed cells were injected into one hemisphere, two further controls were performed. First, the regional specificity of the effect of astrocyte transplantation was assessed by recording cells within the transplanted hemisphere of two animals, just 1 mm lateral to the transplant. This OD histogram does not differ significantly from the one obtained in the control hemisphere (Fig. 2). The local specificity was further detailed by separating cells recorded

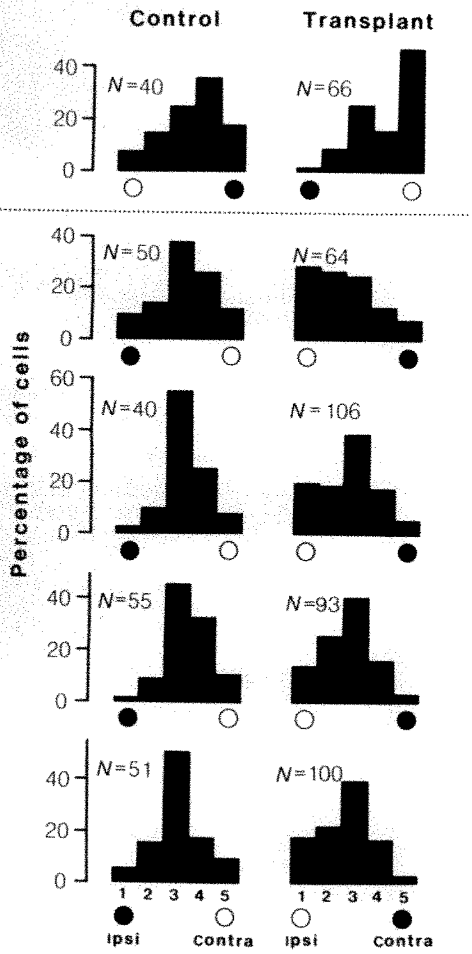
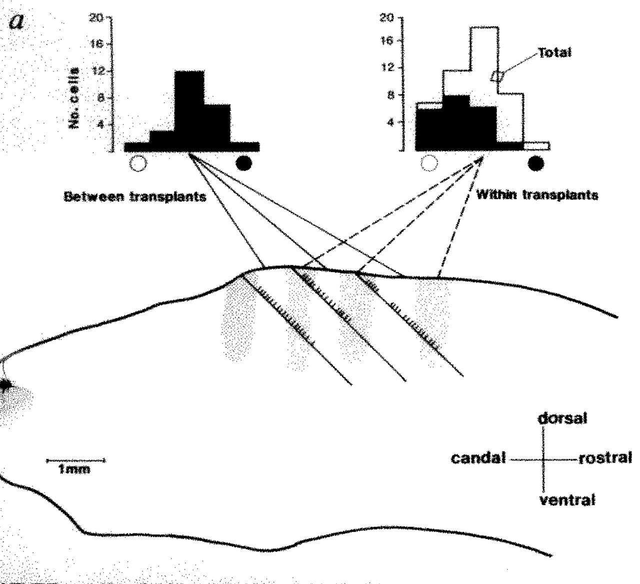


FIG. 3 OD histograms from visual cortex of all five monocularly deprived adult cats that received transplantation of cultured astrocytes. In the control hemispheres (left column), which received previously frozen cell suspension, OD histograms reflect a moderate bias towards the contralateral eye, irrespective of whether this eye was open or closed during the deprivation period (○, open; ●, closed). By contrast, in hemispheres that received intact astrocyte transplantation, OD histograms are always biased towards the open eye. This results in an overproportional presence of contralaterally dominated cells when the ipsilateral eye was sutured closed (row 1), and vice versa (rows 2–5). The overall differences in the OD histograms from the experimental and control hemispheres are statistically significant when tested with the χ^2 test. Calculated significances for each animal from top to bottom are: (1) $\chi^2=28.97$, $P<0.001$; (2) $\chi^2=20.60$, $P<0.001$; (3) $\chi^2=12.23$, $P<0.01$; (4) $\chi^2=31.52$, $P<0.001$; (5) $\chi^2=14.50$, $P<0.01$.



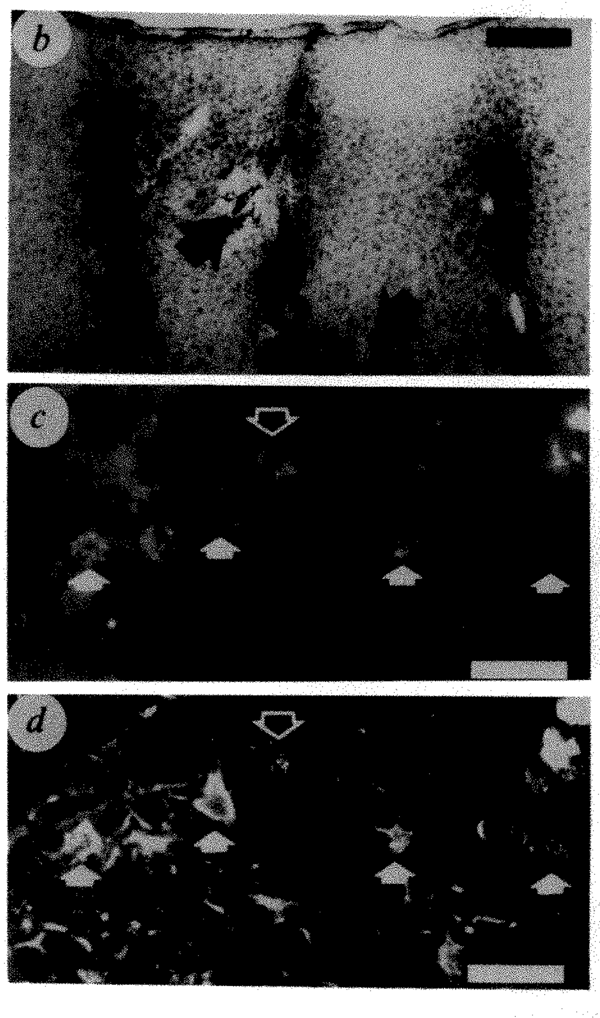


FIG. 4 *a*, OD histograms for cells recorded along the three penetrations passing through locations where transplanted astrocytes were present (dotted). Cells located on the border of the transplants ($N=7$) and two unresponsive units were omitted. OD histograms for cells recorded outside the transplants show a moderate bias towards the contralateral (deprived) eye, whereas a clear bias towards the (experienced) ipsilateral eye is seen in the OD distribution from cells recorded within transplanted regions. The composite histogram of the entire sample of cells given by the open columns in the right histogram continues to show a moderate bias towards the non-deprived eye. *b*, Low-power photomicrograph of the section drawn in *a*, stained with a monoclonal antibody directed against GFAP. Intense immunostaining is present at the sites of astrocytic transplants. The three transplantation sites correspond to rostral transplants in a. Two electrolytic lesions (arrows) mark an electrode track passing through the central transplantation site. *c*, *d*, Higher-power photomicrographs from the border region of an astrocyte transplant viewed with a fluorescent microscope. Transplanted cells were identified by the presence of rhodamine-labelled latex microspheres (*c*); the astrocytic nature of these cells was shown by the presence of GFAP-immunoreactivity (*d*). Note that most rhodamine-labelled cells also display GFAP immunoreactivity (for example, those marked by closed arrows in *c* and *d*). A subpopulation of cells containing rhodamine-labelled particles (open arrow in *c* and *d*) are not GFAP positive and thus may correspond to non-astrocytic cells such as fibroblasts or macrophages. Scale bars: *b*, 500 μm; *c* and *d*, 25 μm.

METHODS. *a*, The location of the transplants was made visible by the presence of cells prelabelled with rhodamine-coupled microspheres fed to the astrocytic cultures (10 μl per 7 ml culture medium; 12 h) before transplantation (see *c*, *d*). Electrode tracks were reconstructed using electrolytic lesions as references (see *b*). *b*, Vibratome sections (50 μm) were incubated with a monoclonal antibody directed against GFAP (Boehringer). Antigen-locations were visualized using the Avidin–biotin method with diaminobenzidine as the chromogen[8]. *c*, *d*, For concurrent visualization of fluorescent latex microspheres, fed to the astrocytic cultures before transplantation, and GFAP, sections were incubated with the monoclonal antibody to GFAP made visible by a fluorescein isothiocyanate-conjugated antibody.

within and outside tranplants. Electrode tracks were reconstructed with reference to small electrolytic lesions (Fig. 4*b*) and matched with transplantation sites made visible by pre-labelling the transplanted cells with fluorescent latex microspheres in two animals. Microscopic observation on sections stained with an antibody against glial fibrillary acidic protein (GFAP)[8], an astrocytic marker, revealed that transplanted astrocytes were localized only in the vicinity of the transplants (Fig. 4). The cells were located in a region displaying an increased immunoreactivity for GFAP (Fig. 4*b*), which reflected the reactive gliosis due to local damage by the injection. Reactive gliosis was found also in regions where non-astrocytic cells and pre-frozen cells had been injected. The OD shift to the non-deprived (ipsilateral) eye was only present in the cell population situated within the transplants (Fig. 4*a*). In a second control experiment, we implanted two additional cats with cultured fibroblasts instead of astrocytes. The OD histogram from the transplanted hemispheres does not differ significantly from those obtained from the control hemispheres, which had received previously frozen cell suspension (Fig. 2).

These data show that the transplantation of astrocytes cultured from the cortices of newborn kittens is sufficient to produce OD changes induced by light deprivation in adult cats. The finding that injection of previously frozen cell suspensions and of cultured fibroblasts does not lead to OD shifts indicates that the transplantation of cells *per se*, or reactive gliosis, which is always associated with brain damage, is not sufficient to induce OD plasticity in adult cats. Reactive gliosis is mediated only to a minor extent by glial proliferation[12,13], and hence may not lead to the generation of a sufficient number of immature astrocytes. In addition, it seems unlikely that macrophages are the relevant cell type in the transplanted cultured cells because (1) the cells constituted only a minor fraction of the cultures (<2%), and (2) a high density of macrophages has to be expected in all transplanted regions because of their massive proliferation and invasion after local cortical damage[14].

How do astrocytes support visual cortical plasticity, specifically axonal sprouting and synapse elimination? Several neurotrophic factors, such as nerve growth factor, basic fibroblast growth factor and the S-100 protein, are known to be synthesized and released by astrocytes[15,16]. The ability to release trophic factors is developmentally regulated[15]. It is conceivable that such factors are released by immature astrocytes, and are selectively taken up by active terminals to support axonal sprouting or neurite extension. Furthermore, synapse elimination could be mediated by active separation of synapses by glial processes, and subsequent phagocytosis of the terminals. Such a mechanism has been observed in the normal development of the spinal chord[17-19]. A similar involvement of astrocytic processes in the regulation of synapses is found in the hypothalamo-neurohypophyseal system[20-23]. Recently it was shown that the increase of synapse density which occurs in glia-free cerebellar cultures is only reversed when astrocytes are transplanted to the cultures[24]. The temporal limitation of synapse elimination to periods when immature astrocytes are present could be due to the loss of the astrocytes' ability during maturation to undergo morphological changes and to perform phagocytosis[25,26].

The data presented here further support the hypothesis that immature glial cells are causally involved in visual cortical plasticity. The mechanisms underlying the experience-dependent change of cortical connectivity discussed above could also explain the beneficial effect of transplanted astrocytes on behavioural recovery after cortical damage[27]. It remains to be shown, however, whether astrocytes actively participate in the plastic changes proper. ☐

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1. Singer, W. in *The Neural and Molecular Bases of Learning* (eds Changeux, J. P. & Konishi, M.) 301-336 (Wiley, Chichester, 1987).
2. Le Vay, S., Stryker, M. P. & Shatz, C. J. *J. comp. Neurol.* **179,** 223-244 (1978).
3. Swindale, N. V., Vital-Durand, F. & Blakemore, C. *Proc. R. Soc.* **B213,** 435-450 (1981).
4. Kleinschmidt, A., Bear, M. F. & Singer, W. *Science* **238,** 355-358 (1987).
5. Rauschecker, J. P. & Hahn, S. *Nature* **326,** 183-185 (1987).
6. Bear, M. F. & Singer, W. *Nature* **320,** 172-176 (1986).
7. Kasamatsu, T. & Pettigrew J. D. *Science* **194,** 206-209 (1976).
8. Müller, C. M. *J. comp. Neurol.* (manuscript submitted).
9. Müller, C. M. *Glia* (manuscript submitted).
10. Hubel, D. H. & Wiesel, T. N. *J. Neurophysiol.* **28,** 1041-1059 (1965).
11. Levitt, F. B. & van Sluyters, R. C. *Devl Brain Res.* **3,** 323-327 (1982).
12. Ferrara, N., Ousley, F. & Gospodarowicz, D. *Brain Res.* **462,** 223-232 (1988).
13. Furukawa, S., Furukawa, Y., Satoyoshi, E. & Hayashi, K. *Biochem. biophys. Res. Commun.* **13** 57-63 (1986).
14. Miyake, T., Hattori, T., Fukuda, M. & Kitamura, T. *Brain Res.* **489,** 31-40 (1989).
15. Rudge, J. S., Manthorpe, M. & Varon, S. *Devl Brain Res.* **19,** 161-172 (1985).
16. van Eldik, L. J. & Zimmer, D. B. *Brain Res.* **436,** 367-370 (1987).
17. Ronnevi, L. O. & Conradi, S. *Brain Res.* **80,** 335-339 (1974).
18. Ronnevi, L. O. *J. Neurocytol.* **6,** 487-504 (1977).
19. Ronnevi, L. O. *Cell Tiss. Res.* **189,** 203-217 (1978).
20. Tweedle, C. D. & Hatton, G. I. *Cell Tiss. Res.* **18,** 59-72 (1977).
21. Tweedle, C. D. & Hatton, G. I. *Brain Res.* **309,** 373-376 (1984).
22. Perlmutter, L. S., Tweedle, C. D. & Hatton, G. I. *Neuroscience* **12,** 503-511 (1984).
23. Perlmutter, L. S., Tweedle, C. D. & Hatton, G. I. *Neuroscience* **13,** 769-779 (1984).
24. Meshul, C. K., Seil, F. J. & Herndon, R. M. *Brain Res.* **402,** 139-145 (1987).
25. Skaper, S. D., Facci, L., Rudge, J., Katoh-Semba, R., Manthorpe, M. & Varon, S. *Devl Brain Res.* **25** 21-31 (1986).
26. Saad, Y. A., Al-Zuhair, A. G. H. & Dawod, B. *Glia* **1,** 211-218 (1988).
27. Kesslak, J. P., Nieto-Sampedro, M., Globus, J. & Cotman, C. W. *Expl Neurol.* **92,** 377-390 (1986).
28. McCarthy, K. D. & de Vellis, J. *J. Cell Biol.* **85,** 890-902 (1980).

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Target size regulates calibre and myelination of sympathetic axons

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AXONS in vertebrate peripheral nerves are ensheathed by Schwann cells. For some axons, this sheath consists of a single layer of glial cell cytoplasm and plasma membranes; for other axons, Schwann cells form multilayered myelin. Whether or not a Schwann cell makes myelin is determined by a signal from the axon[1,2], but the nature of this signal is not known. Here I show that sympathetic postganglionic axons, which are normally not myelinated, become myelinated when their calibre is increased as a result of increasing the size of the peripheral target they innervate. This result implies that axon calibre, which is known to be correlated with myelination[3], is in fact the crucial determinant of whether an axon becomes myelinated. Furthermore, the finding that increasing or decreasing target size causes corresponding increases or decreases in axon size indicates that axon calibre is itself regulated by retrograde signals from peripheral target tissues.

To study the effect of peripheral target size on ganglion cell axons, the relative size of a peripheral target of the superior cervical ganglion was altered during development by changing the ratio between the amount of target tissue and the number of innervating nerve cells in the ganglion. The target tissue tested in these experiments was the rat submandibular salivary gland. To make a smaller target than normal, the submandibular duct was ligated in 4-week-old rats; this results in glands weighing approximately 20% of normal when the animals reach 30 weeks of age[4-6]. These smaller glands were innervated by a normal number of superior cervical ganglion neurons (3,800 axons on average). In a second group of rats, relative target size was increased by cutting one branch of the sympathetic nerve innervating the gland at birth. When these animals were examined at 30 weeks of age, the partially denervated glands were of normal size, but were innervated by only 5-50% the normal number of sympathetic neurons[6].

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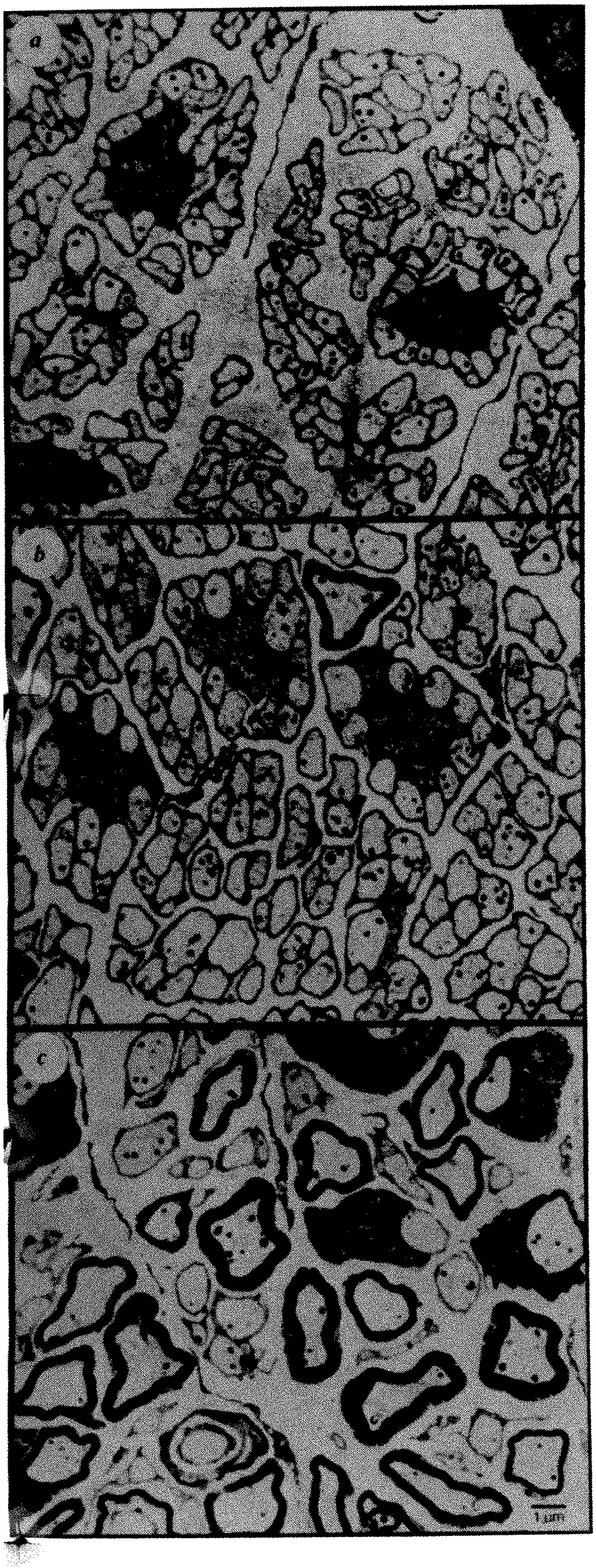

◀ FIG. 1 Effect of target size on the calibre of postganglionic sympathetic axons innervating the submandibular gland. Axons innervating: *a*, small target (submandibular duct ligated at 4 weeks of age); *b*, control target; *c*, large target (gland partially denervated at birth). All determinations were made in adult animals (30 weeks). Mean axon diameters were: *a*, 0.80 ± 0.01 μm; *b*, 1.15 ± 0.01 μm; *c*, 1.43 ± 0.02 μm. A portion of nerve midway between the gland and the external carotid nerve trunk was removed from 6-month-old animals after death; nerves were fixed by immersion and processed for electron microscopy as previously described[6]. Axon circumference was measured from electron micrographs (4,000×) using a digitizing tablet, and axon diameters were calculated as circumference/π. Scale bar is the same for all 3 panels.

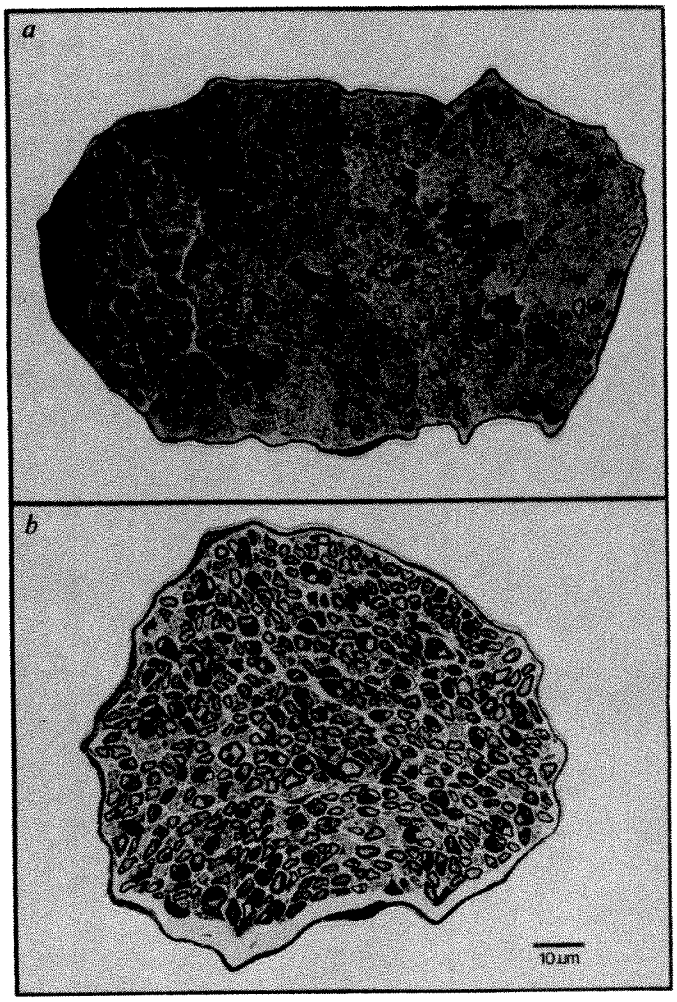

FIG. 2 Effect of increasing target size on the myelination of sympathetic axons. Electron microscope photomontages of whole postganglionic nerves innervating the submandibular gland in *a*, control (sham operated) nerve and *b*, nerve innervating relatively enlarged target (partially denervated at birth); both nerves are shown at the same magnification. In *a*, there are 3,192 unmyelinated and 25 myelinated axons; in *b*, 304 axons are unmyelinated and 386 are myelinated. Nerves were prepared for electron microscopy as described in the legend to Fig. 1. Retrograde labelling of the postganglionic nerve with horseradish peroxidase confirmed that at least 95% of the axons seen in cross-sections were axons of superior cervical ganglion neurons[6].

The postganglionic nerves carrying sympathetic axons to the submandibular gland were cut in cross-section and examined by electron microscopy (Fig. 1). Decreasing relative target size resulted in axons whose diameters were on average 31% (±0.5% s.e.m.) smaller than control axons, whereas increasing relative target size resulted in axons whose diameters were 24% (±1.7%) larger than normal. These changes in axon diameter are comparable to the magnitudes of changes previously observed in cell soma diameter and dendritic length when target size is altered under similar experimental conditions[6]. Taken together, these observations suggest that retrograde signals from the target provide a general mechanism for regulating the size of superior cervical ganglion neurons and their processes.

Target-induced changes in the size of the axon were associated with significant changes in the way in which these axons were

TABLE 1 Effect of axonal target size and preganglionic denervation on Schwann cells associated with postganglionic sympathetic axons

Experimental condition	Number of animals	Target size (μg/axon)	Total number of axons	Number of myelinated axons	Total number of Schwann cells/ cross-section
Small target	3	24 ± 4	3,596 ± 102	13 ± 1	323 ± 19
Control	4	90 ± 4	3,777 ± 90	24 ± 4	426 ± 39
Large target A	3	252 ± 56	1,620 ± 396	440 ± 189	1,111 ± 352
Large target B	2	532 ± 27	636 ± 38	291 ± 67	470 ± 75
Large target C	2	1,463 ± 155	233 ± 15	140 ± 1	233 ± 15
Preganglionic denervation					
Control target	2	73 ± 3	3,971 ± 458	19 ± 5	401 ± 22
Large target	2	954 ± 506	595 ± 292	141 ± 57	—

In the top part of the table, animals with relatively large targets were divided into 3 groups (A–C) because of the wide range of sizes produced by partial denervation (see text). Data in the lower part of the table show the effect of partial denervation of the submandibular gland for animals in which the preganglionic nerve was cut at birth[6]. The number of Schwann cells in a cross section of the nerve was estimated by dividing the total number of axons in the nerve by the number of axons per Schwann cell (calculated as described in Fig. 3.) Values are given ±s.e.m.

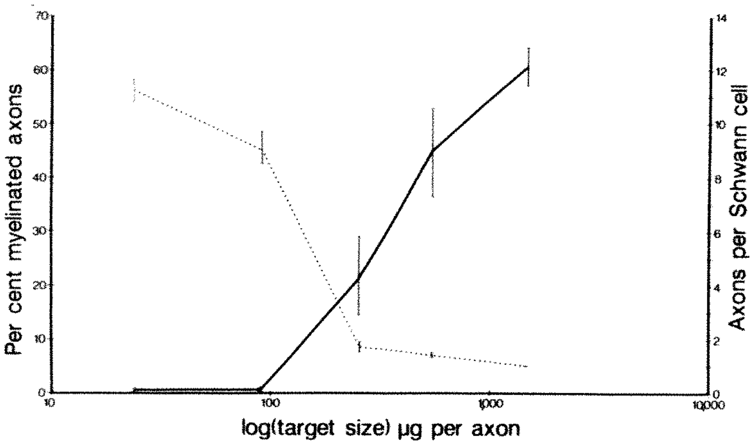

FIG. 3 Influence of target size on myelination, expressed as the percentage of axons that are myelinated (unbroken line), and on the number of axons ensheathed by each Schwann cell (dotted line). Target size is defined as the mass of the submandibular gland divided by the number of innervating sympathetic axons (counted in photomontages of whole nerves, as in Fig. 2). Each data point is the mean of values from several animals (Table 1). The number of axons ensheathed per Schwann cell was counted for each glial cell whose nucleus was visible in the plane of section; axon profiles in direct contact with the Schwann cell membrane were considered to be ensheathed by that glial cell.

ensheathed by Schwann cells. In control animals, 99.3% (±0.1% s.e.m.) of the axons in the postganglionic nerve were unmyelinated. When relative target size was increased by partial denervation, there was a dramatic increase in the number of myelinated axons in the nerve (Fig. 2). The absolute number of myelinated axons increased twelve times, on average, over the number in control nerves. Moreover, the fraction of axons that was myelinated increased progressively with increasing size of the peripheral target; for the largest targets, 60% of the axons were myelinated (Table 1; Fig. 3). When postganglionic nerves innervating experimentally enlarged targets were examined at multiple sites between the ganglion and the gland, the number of myelinated axons counted at each level was significantly above the number present in controls, indicating that these axons were myelinated along their entire length. Finally, an increase in myelination could be induced by increasing target size even if the superior cervical ganglion was denervated at birth (Table 1), suggesting that the increased myelination does not depend on electrical activity in the ganglion cells.

Two lines of evidence in this study indicate that the signal for Schwann cells to myelinate sympathetic axons is an increase in axon calibre. First, in agreement with previous reports[3,7,8], there was a clear size threshold above which axons became myelinated. Whereas 95% of unmyelinated axons were less than 1.6 μm in diameter, regardless of the experimental conditions, 90% of myelinated axons were greater than 1.6 μm in diameter. Second, even for axons below this size threshold for myelination, the number of axons ensheathed by each Schwann cell varied systematically with target size and axon calibre. Thus, when axon calibre was decreased by making a smaller target, there was no significant change in the number of myelinated axons in the postganglionic nerve, but there was a significant increase in the average number of axons ensheathed by each glial cell. On the other hand, as target size and axon calibre were increased in these experiments, the average number of axons associated with each glial cell gradually decreased (Fig. 3). This decrease was accompanied by an increase in the total number of Schwann cells present in the nerve (Table 1).

These systematic changes in the way in which Schwann cells ensheath axons of different sizes imply that Schwann cells are capable of responding to changes in axon calibre long before the axons reach the size threshold for myelination. Interestingly, the point at which Schwann cells switched from simple ensheathment to myelination was the point at which the ratio of axons to Schwann cells approached unity (Fig. 3). This relationship suggests that a Schwann cell that does not produce myelin might be triggered to begin myelination not because it detects some critical axon diameter, but rather because it reaches a point where it can no longer accommodate further axon growth by reducing the number of axons ensheathed. After Schwann cells switch to a myelinating phenotype, they once again respond to axon growth in a graded manner. In this case, however, the response is not to relinquish axons, but to add additional layers of compact myelin[7].

These results imply that there are no qualitative differences between axons that normally become myelinated and those that do not: increasing the size of a normally unmyelinated axon is sufficient to induce Schwann cells to myelinate it. These experiments also show that the calibre of peripheral axons is regulated by the size of their target. This regulation provides a mechanism by which the nervous system can continuously adjust to the growth of target tissues by modifying axonal conduction properties through retrograde signals from the target; these signals affect both axon size and myelination. □

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1. Weinberg, H. J. & Spencer, P. S. *Brain Res.* **133**, 363–378 (1975).
2. Aguayo, A. J. *et al J. Neurocytol.* **5**, 565–573 (1976).
3. Duncan, D. *J. comp. Neurol.* **60**, 437–472 (1934).
4. Tamarin, A. in *Secretory Mechanisms of Salivary Glands* (ed. Schneyer, L. H. & Schneyer, C. A.) 220–237 (Academic, New York, 1966).
5. Womble, M. D. & Roper, S. *J. Neurophysiol.* **58**, 276–287 (1987).

6 Voyvodic J T *J Neurosci* **9,** 1997-2010 (1989)
7 Sanders F K *Proc R Soc* **B135,** 323-357 (1948)
8 Friede R & Samorojski T *J comp Neurol* **130,** 223-231 (1967)

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Membrane depolarization evokes neurotransmitter release in the absence of calcium entry

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THE discovery that Ca^{2+} is necessary for the release of neurotransmitter, the primary means by which nerve cells communicate, led to the calcium hypothesis of neutransmitter release[1–4], in which release is initiated after an action potential only by an increase in intracellular Ca^{2+} concentration near the release sites and is terminated (1–2 ms) by the rapid removal of Ca^{2+}. Since then, the calcium-voltage hypothesis has been proposed[5,6], in which the depolarization of the presynaptic terminals has two functions. First, in common with the calcium hypothesis, the Ca^{2+} conductance is increased, thereby permitting Ca^{2+} entry Second, a conformational change is induced in a membrane molecule that renders it sensitive to Ca^{2+}, and then binding of Ca^{2+} to this active form triggers release of neurotransmitter. When the membrane is repolarized, the molecule is inactivated and release is terminated, regardless of the local Ca^{2+} concentration at that moment. This hypothesis, in contrast to the calcium hypothesis, accounts for the insensitivity of the time course of release to experimental manipulations of intracellular Ca^{2+} concentation[7–11] Furthermore, it explains rapid termination of release after depolarization, even though Ca^{2+} concentration may still be high. Here we describe experiments that distinguish between these two hypotheses and find that our results support the calcium voltage hypothesis.

A critical test to distinguish the two hypotheses for the mechanism of neurotransmitter release separate the two effects of depolarization by raising intracellular Ca^{2+} and inducing membrane depolarization without entry of Ca^{2+} The calcium hypothesis would then predict that the membrane depolarization would not increase release, whereas the calcium-voltage hypothesis would predict an increase in release, locked in time to the depolarizing pulse The introduction of photosensitive Ca^{2+} chelators, the 'caged Ca^{2+}' compounds[12], makes such tests feasible These compounds have a high affinity for Ca^{2+}, but after exposure to light, the affinity is reduced and Ca^{2+} is released It is therefore possible to induce a phasic increase of Ca^{2+} concentration inside a presynaptic terminal without changing the membrane potential

Zucker and Haydon[13] used a caged Ca^{2+} compound, nitr-5, to test the calcium-voltage hypothesis, and found that asynchronous transmitter release was induced by photolysis of nitr-5 In the absence of Ca^{2+} entry, as depolarization did not affect this asynchronous release, it was concluded that membrane depolarization has no direct role in the release process The experiments underlying this conclusion, however, were made on a synapse in culture, where release is slow It is possible that, unlike in fast synapses, a change in membrane potential does not play a part in determining the time course of release, and/or that the experimental protocol was not designed to bring out such effects (see our delay histogram, Fig 3) We used nitr-5 to test the calcium-voltage hypothesis at a conventional fast synapse, the neuromuscular junction of the opener muscle in the first walking leg of the small crayfish *Procambarus clarkii* It has been indicated that in this preparation membrane potential has a role in addition to the classical one of opening Ca^{2+} channels[5,14]

Figure 1 depicts the time course of the effects of pressure-injecting nitr-5 into the excitatory axon through an intracellular microelectrode placed close to the terminal[15] Baseline responses to twin impulses (20 ms apart) recorded shortly after the intracellular penetration are shown in Fig 1*a* A reduction of release was apparent within three minutes after the start of injection (Fig 1*b*) As time elapsed and more nitr-5 was injected into the axon, release was further reduced (Fig 1*c*) until, after 18 min, it was nearly abolished (Fig 1*d*) This reduction in release is expected because nitr-5 chelates Ca^{2+} and its K_D in darkness is close to the resting level of intracellular Ca^{2+} ($\sim$0 1 μM) These results confirm that nitr-5 is reaching the nerve terminals At this state, the frequency of stimulation was raised to load the terminal with Ca^{2+} and to allow release to be measurable (Fig 2*a*) A flash of light was then delivered which resulted in a transient threefold elevation of the evoked release (Fig 2*b*) About one second after the flash, release is stabilized at a level higher than the control (compare Fig 2*a* with Fig 2*b*), indicating that the increase of intracellular Ca^{2+} concentration achieved by photolysis of nitr-5 is in a range that is effective for release

Given these results, nitr-5 could be directly used to test the unique feature of the calcium-voltage hypothesis To this end, we blocked Ca^{2+} entry using an extracellular solution essentially devoid of Ca^{2+} Addition of EGTA damages the preparation, so we added a high concentration of Mg^{2+} (12 5 mM) both to block any possible entry of Ca^{2+} and also to substitute for Ca^{2+} in opening postsynaptic glutamate channels[16] The effect of

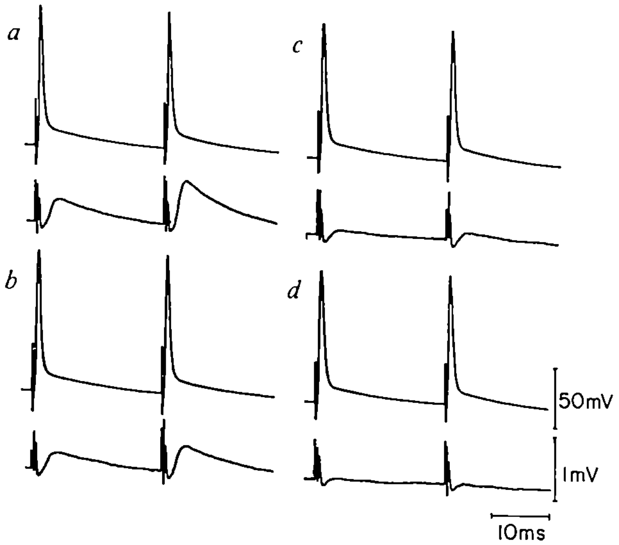

FIG 1 *a–d*, Pressure-injection of nitr-5 reduces synaptic release Upper traces show the axon action potential Lower traces show the synaptic potentials Twin impulses, 20 ms apart, were given at 3 3 Hz Each record is an average of 50 sweeps *a*, Control, before nitr-5 injection, and *b*, 3 min, *c*, 10 min and *d*, 18 min after beginning of injection of nitr-5 The excitatory axon was impaled distally to the main bifurcation[15] by an intracellular microelectrode, which served for recording and for pressure injection of nitr-5 The axon was stimulated by a suction electrode at its entry to the opener muscle Another intracellular electrode recorded synaptic potentials from a muscle fibre between these two sites

METHODS Crayfish were supplied by Atchafalaya, Louisiana The first walking legs of small (body length 4–4 5 cm) animals were used The bevelled microelectrode contained 50 mM nitr-5 (tetrasodium salt, Calbiochem), 35 mM $CaCl_2$, 85 mM KCl, 10 mM Tris-HCl, pH 7 4, and 0 5% fast green For pressure injection (1 s, 10–40 psi) the Pico-Injector Model PLI-100 (Medical System) was used Between 40–100 injection pulses were given during a period of ~30 min E p s ps were recorded with a microelectrode containing 2 5 M potassium acetate (6–10 MΩ) The preparation was perfused with a solution containing 220 mM NaCl, 5 4 mM KCl, 12 mM $CaCl_2$, 2 5 mM $MgCl_2$, 10 mM Tris-maleate, pH 7 6, at 18–20 °C Transilluminating light was filtered to remove wavelengths below 475 nm to avoid nitr-5 photolysis voltage on the probability of release was assessed by constructing synaptic delay histograms[3,5] After the injection of nitr-5 and perfusion with the Ca^{2+}-entry blocking solution, evoked release was abolished That is, miniature endplate potentials (m e p ps) were about equally distributed 10 ms before and 10 ms after stimulus pulses (Fig 3*b*) Then, while the nerve was being stimulated, three flashes of light (about 40 s apart) were given The results were pooled in a histogram (Fig 3*c*) Two changes occurred (compare Fig 3*b* and *c*) First, the baseline level of spontaneous m e p ps increased by more than 6 times Second, an additional increase in the rate of release was observed in the time range 2–4 ms after the stimulus That is, there was now evoked release Furthermore, the time range of this evoked release was consistent with the time course of release in the presence of extracellular Ca^{2+} (refs 5, 14) We obtained similar results in four further experiments

The increase in transmitter release after each flash was transient For example, delay histograms constructed from the first 200 impulses (10 s) following the first flash contained 7 m e p ps (in 10 ms following the stimulus), whereas the next 200 impulses resulted in only 2 m e p ps The transient increase in release indicates that after each flash the intracellular Ca^{2+} concentration increased briefly and that the Ca^{2+} was then removed by the intracellular Ca^{2+}-buffering systems The observed evoked release could have been due to a temporary elevation of intracellular Ca^{2+} concentration Alternatively, it could have resulted from a reduced ability of the photolysed nitr-5 to chelate small amounts of Ca^{2+} that might have entered the terminal As the reduction in the affinity of the photolysed nitr-5 is not reversible but the increased release was transient we favour the first possibility

The level of stimulus-evoked release in our experiments was rather lower than that seen in the presence of extracellular Ca^{2+} It is possible that intracellular Ca^{2+} buffers compete with the nitr-5 to strip away some of its Ca^{2+}, diminishing the increase in Ca^{2+} after a flash We therefore examined the effect of interfering with intracellular Ca^{2+}-buffering mechanisms using the uncoupler carbonyl cyanide *m*-chlorophenylhydrazone (CCCP)[17] Two additional modifications were made in this experiment First, 0 2 mM Ca^{2+} was added to the bathing medium to improve the ability of the axon to sustain action potentials Second, as there was a small amount of Ca^{2+} in the medium, the measures for blocking Ca^{2+} entry were strengthened by including 2 mM Mn^{2+} in addition to the 12 5 mM Mg^{2+} In the controls without nitr-5 injection, release could not be evoked at stimulation rates of 10 Hz In 1,000 pulses, only six quanta were seen This was so even in control trials where 3,4-diaminopyridine (0 1 mM) was added and the nerve action potential was clearly broadened Figure 4*a* (traces 1 and 2, fast and slow time base, respectively) shows synaptic responses in the presence of extracellular Ca^{2+} 75 minutes after injection of nitr-5 Figure 4*b* shows the block of release in the Ca^{2+}-blocking medium The responses seen in Fig 4*c* were obtained 11 minutes after application of 10 μM CCCP Two effects are evident First, there was increase in the spontaneous release (Fig 4*c*, trace 2), presumably resulting from an increase in intracellular Ca^{2+} concentration The more striking result, however, was the appearance of synaptic potentials These are seen even after single impulses without averaging Now after a flash of light (Fig 4*d*), the amplitude of evoked excitatory postsynaptic potentials (e p s ps) and the frequency of spontaneous m e p ps increased even more

Interestingly, Zucker and Lando[17], who applied 10 μM CCCP in the same preparation, did not observe evoked release The difference between their results and ours may be explained by

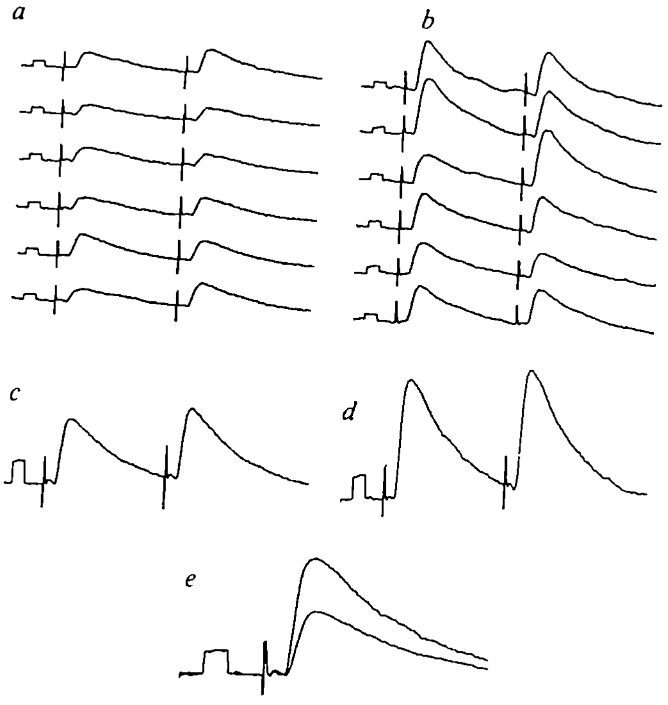

FIG 2 Photolysis of nitr-5 caused an increase in evoked release This is a continuation of the experiment shown in Fig 1 Twin-impulse stimulation (20 ms intervals) at 10 Hz slowly restored release (compare with Fig 1*d*) *a*, Single responses (sampled at 5 Hz) after e p s ps reached a steady-state level *b*, Single responses after a flash of light (top to bottom) *c*, An average of 10 sweeps before the light flash *d*, An average of 5 sweeps after the light flash *e*, Superposition of the first responses in *c* and *d* Calibration pulse 1 mV, 2 ms

METHODS The xenon lamp was an ensemble of a Strobex Power Pack, model 238 and a Strobex Lamp model 278 (Chadwick-Helmuth, California) The flash lamp was mounted in one of two configurations In the first, the lamp was aimed with an angle of 45°, in the second it was positioned vertically over the preparation The light was filtered to remove short-wave ultraviolet (<305 nm) The energy of the flash was about 180 joules for the first configuration and 100 joules for the second A light flash without injection of nitr-5 had no effect The light was focused with an elliptical mirror (Pichel, California) to give an image of ~7 mm at the focal plane

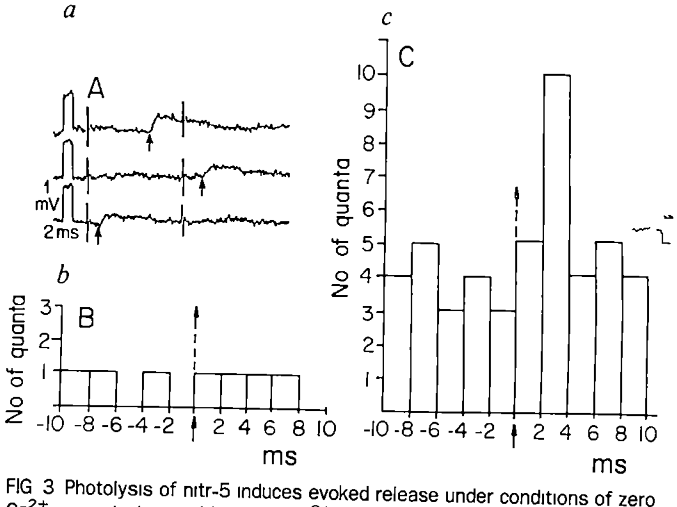

FIG 3 Photolysis of nitr-5 induces evoked release under conditions of zero Ca^{2+} concentration and 12 5 mM Mg^{2+} in the external medium Twin impulses (20 ms apart) were given at a repetition rate of 10 Hz The delay from the stimulus to a quantum response was measured (arrows in *a*) Delay histograms (bin size 2 ms) describing the probability of occurrence of single events are given for 10 ms before the stimulus (negative timescale) and after the stimulus Responses were measured for a period of 10 ms after the two impulses in a sweep and lumped together For responses appearing before stimulation, measurements were taken for two successive periods of 10 ms before the first stimulus and lumped together *a*, Examples of records after a flash of light *b*, Delay histogram after injection of nitr-5 in the dark *c*, Delay histogram in a nitr-5 loaded preparation following 3 consecutive flashes of light For each histogram, 600 sweeps were taken, the histogram contains responses to 1,200 impulses Arrows in *b* and *c*, indicate point of stimulation

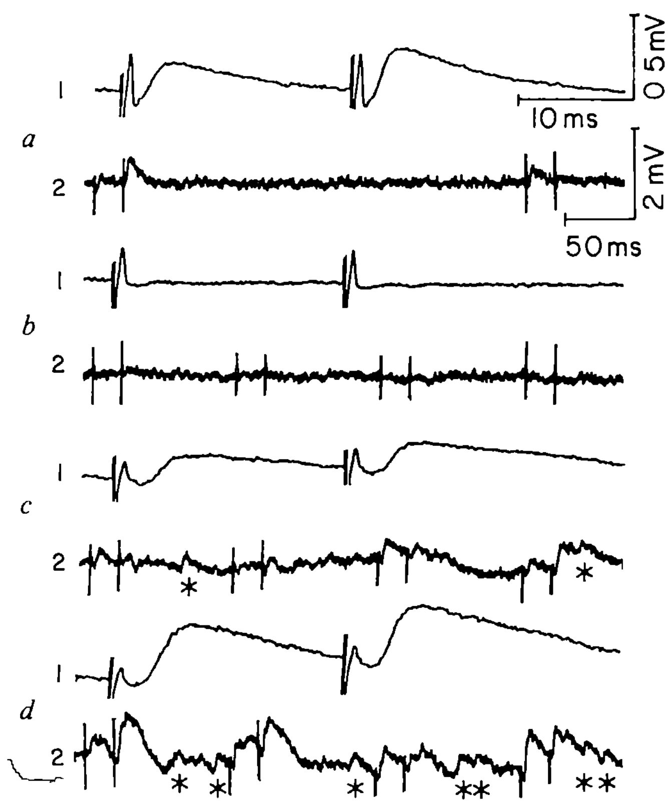

FIG 4 Ca^{2+} loading of the nerve terminal supports action potential evoked release in the absence of Ca^{2+} entry *a*, Trace 1 is an average of 100 traces taken at 3 3 Hz 75 min after the injection of nitr-5, trace 2 is a single trace, at a slow time base, showing evoked release but not spontaneous release *b*, Records taken 4 min after wash with a solution containing 0 2 mM Ca^{2+}, 12 5 mM Mg^{2+} and 2 mM Mn^{2+} The wash was very rapid and effective as the washing solution was directly aimed at the small space of the opener muscle in the shell It is shown in trace 1 that evoked release is completely blocked, even at 10 Hz simulation rate and in trace 2 that no m e p ps are seen Eleven minutes after addition of 10 μM CCCP, evoked release can be seen in the average record in *c* (trace 1) and m e p ps (asterisks) in trace 2 The single trace (2) in *c* also shows evoked e p s ps *d*, After a flash of light, a further increase in the size of the evoked response is evident in trace 1 (average of 50 traces), with a concomitant increase in m e p ps frequency in trace 2 In the averaged responses in *a*(1)–*d*(1), the nerve action potential appearing before the synaptic potential originates from cross talk between the two microelectrodes

two factors (1) their measurements were taken too long after the application of CCCP, and (2) the injection of nitr-5 might have postponed the build-up of a toxic level of Ca^{2+} concentration because of its Ca^{2+}-chelating properties It is known that at a high intracellular Ca^{2+} concentration, evoked release is blocked while spontaneous release continues[18] Indeed, we measured release shortly after application of CCCP, when the frequency of spontaneous m e p ps was still low

We conclude that (1) after a flash of light in nitr-5-injected axons, particularly in the presence of CCCP, spontaneous release is enhanced, these m e p ps appearing randomly in time, (2) under conditions of raised intracellular Ca^{2+}, depolarizing impulses evoked synchronous release, despite the blocking of Ca^{2+} entry, and (3) the time course of this evoked release was normal Therefore, in the fast synapse of the crayfish neuromuscular junction, membrane depolarization plays a part in the release process, in addition to its well-known effect of opening Ca^{2+} channels ☐

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1 Katz B & Miledi R *Proc Roy Soc* **B161**, 496–503 (1965)
2 Miledi R *Proc Roy Soc* **B183**, 421–425 (1973)
3 Katz B & Miledi R *Proc. Roy Soc* **B161**, 483–495 (1965)
4 Smith S U & Augustine G J *Trends Neurosci* **11**, 458–464 (1988)
5 Parnas I Parnas H & Dudel J *Pflügers Arch Eur J Physiol* **406**, 131–137 (1986)
6 Parnas H, Parnas I & Segel L A *Int Rev Neurobiol* (in the press)
7 Barrett E F & Stevens C F *J Physiol Lond* **227**, 691–708 (1972)
8 Matzner H, Parnas H & Parnas I *J Physiol Lond* **398**, 109–121 (1988)
9 Parnas H, Hovav G & Parnas I *Biophys J* **55**, 859–874 (1989)
10 Datyner N B & Gage P W *J Physiol Lond* **303**, 299–314 (1980)
11 Andreu R & Barett E F *J Physiol Lond* **308**, 79–97 (1980)
12 Adams S R Kao J P Y Grynkiewicz G Minta A & Tsien R Y *J Am chem Soc* **110**, 3212–3220 (1988)
13 Zucker R S & Haydon P G *Nature* **335**, 360–362 (1988)
14 Parnas H Dudel J & Parnas I *Pflugers Arch Eur J Physiol* **496**, 121–130 (1986)
15 Wojtowicz J M & Atwood H L *J Neurobiol* **14**, 385–390 (1983)
16 Hatt H Franke C H & Dudel J *Pflugers Arch Eur J Physiol* **411**, 17–26 (1988)
17 Zucker R S & Lando L *Science* **231**, 574–579 (1986)
18 Adams O J Takeda K & Umbach J A *J Physiol Lond* **369**, 145–159 (1985)

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Germ-line transmission of a disrupted β_2-microglobulin gene produced by homologous recombination in embryonic stem cells

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MAJOR histocompatibility complex (MHC) class I molecules are integral membrane proteins present on virtually all vertebrate cells and consist of a heterodimer between the highly polymorphic α-chain and the β_2-microglobulin (*β2-m*) protein of relative molecular mass 12,000 (ref 1) These cell-surface molecules play a pivotal part in the recognition of antigens, the cytotoxic response of T cells, and the induction of self tolerance[1,2] It is possible, however, that the function of MHC class I molecules is not restricted to the immune system, but extends to a wide variety of biological reactions including cell–cell interactions For example, MHC class I molecules seem to be associated with various cell-surface proteins, including the receptors for insulin, epidermal growth factor, luteinizing hormone and the β-adrenergic receptor[3–6]. In mice, class I molecules are secreted in the urine and act as highly specific olfactory cues which influence mating preference[7,8]. The *β2-m* protein has also been identified as the smaller

TABLE 1 Frequency of gene targeting in D3 cells

Expt	PCR$^+$ pools*	PCR$^+$ pools confirmed by cell cloning*	G418^r colonies†	Targeted clones/total clones	Estimated targeting frequency‡
1	7/32	3/3	15	5/31	1 in 30
2	19/28	4/4	36	5/40	1 in 16

ES cells were electroporated with the targeting vector and selected for G418^r as described in Fig 2

* Expressed per total number tested

† Average number per 48-well plate

‡ The frequency of targeting was calculated using the Poisson distribution formula, in which the fraction of plates with no recombinants, *P*(0) is given by e^{-m}, where *m* is the average number of recombinants per pool ($m = -\ln P(0)$) The targeting efficiency is the ratio of *m* to the average number of independent G418^r clones per pool, which is half the observed number based on the assumption that each pool has on average two sibling clones PCR$^+$, scored positive by PCR

component of the Fc receptor in neonatal intestinal cells[9], and it has been suggested that the protein induces collagenase in fibroblasts[10]. Cells lacking *β2-m* are deficient in the expression of MHC class I molecules, indicating that the association with *β2-m* is crucial for the transport of MHC class I molecules to the cell surface[1]. The most direct means of unravelling the many biological functions of *β2-m* is to create a mutant mouse with a defective *β2-m* gene We have now used the technique of homologous recombination to disrupt the *β2-m* gene. We report here that introduction of a targeting vector into embryonic stem cells resulted in *β2-m* gene disruption with high frequency. Chimaeric mice derived from blastocysts injected with mutant embryonic stem cell clones transmit the mutant allele to their offspring

Pluripotent embryonic stem cells can be genetically manipulated in culture, and when introduced into a host blastocyst, contribute to all tissues of the chimaeric mouse[11,12] The contribution of an embryonic stem (ES) cell carrying a defined mutation to the germ line of the chimaera thus results in a new mouse strain carrying the mutation Gene targeting, mediated by recombination between a vector and homologous chromosomal sequences, has been used in ES cells to disrupt several genes, including the hypoxanthine-guanine phosphoribosyl transferase gene (*HPRT*)[13-15], the *int-2* proto-oncogene[14], the mouse *engrailed*-like gene *En-2* (ref 16) and the *adipson*, the aP2 and the *fos* gene[25] In these cases, the targeting vector contained the bacterial neomycin-resistance gene (*neo*^{r}) as a selectable marker gene facilitating the isolation of targeted clones More recently, the herpes simplex virus (HSV) thymidine kinase gene (*tk*) has been included in the vector to permit negative selection agains nonhomologous integration events[14] An alternative strateg using microinjection of a mutated gene fragment has been use to disrupt the *Hox1 1* gene in ES cells[17] Recently, germ-lir transmission of an *HPRT* mutant gene corrected by gene targeting has been reported[18], confirming that gene disruption in E cells can represent a general approach to generate defined mutations in mice

To mutate the *β2-m* gene, a replacement-type vector wa designed which combined several previously used characteristics[13,14] facilitating the detection of targeting events The vecto contained 10 kilobases (kb) of homology to the *β2-m* gene, *neo*^{r} cassette in the second exon lacking a polyadenylation sit and a *tk* gene at the 5′ end (Fig 1*a*) Initial Southern-blo analyses revealed, however, that 50% of randomly picked G418 resistant (Neo^r) ES cell clones had lost the *tk* gene Therefore no counter selection against non-homologous integration event was applied in our experiments

The following screening procedure was devised to allow rapid identification of targeted clones (Fig 2) D3 ES cells[12] were electroporated on day 0, divided into ~30 independent pools and grown for 3 5 days in normal medium, by which time a cell containing a targeting event had divided into ~8 daughter cells At that time, the pools of cells were split in half and subjected to drug selection One-half of each pool (each containing 15-40 G418^r colonies after plating) was grown for 10 days as a bulk culture and used for polymerase-chain-reaction (PCR) analysis to identify the presence of a targeted cell The other half of each

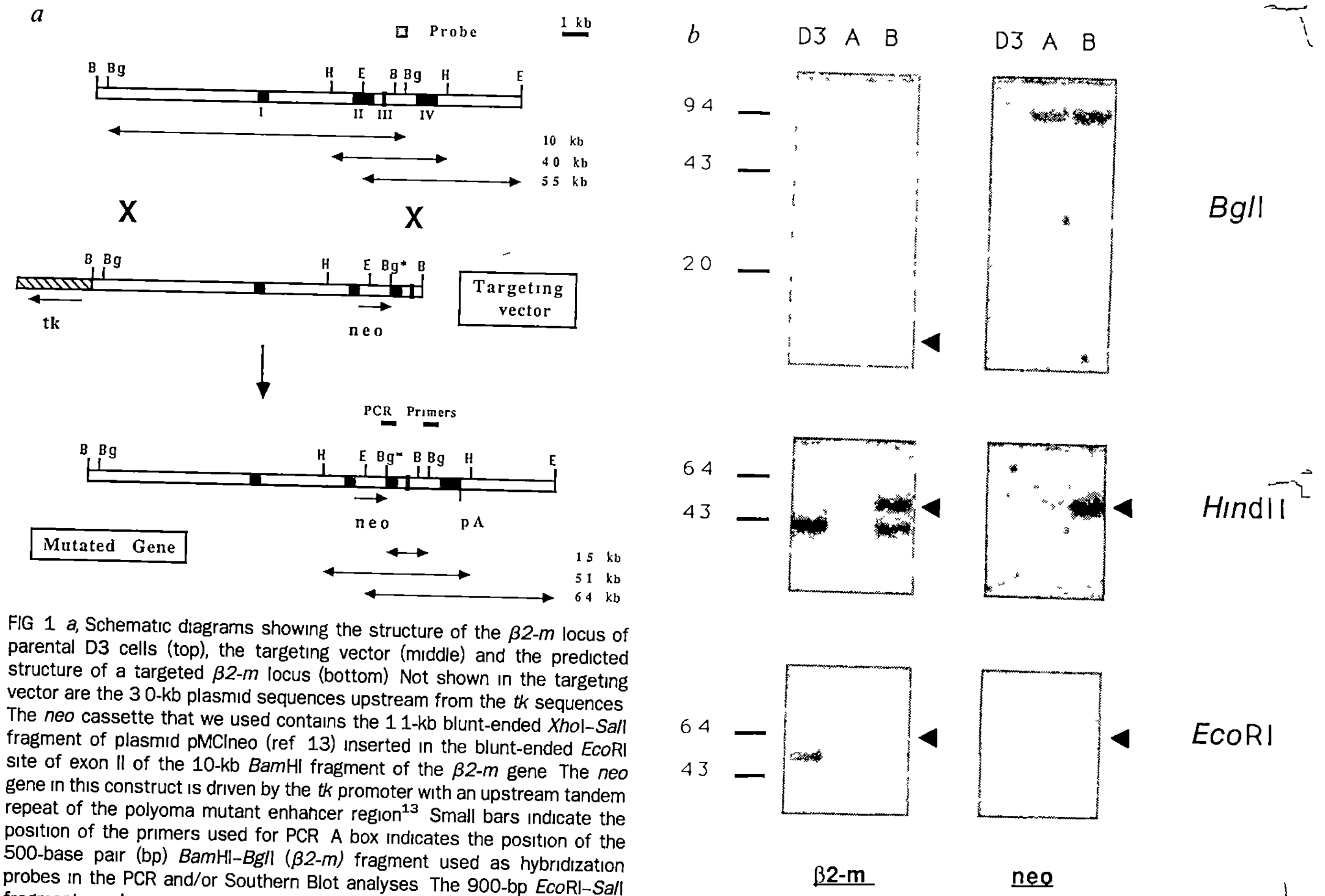

FIG 1 *a*, Schematic diagrams showing the structure of the *β2-m* locus of parental D3 cells (top), the targeting vector (middle) and the predicted structure of a targeted *β2-m* locus (bottom) Not shown in the targeting vector are the 3 0-kb plasmid sequences upstream from the *tk* sequences The *neo* cassette that we used contains the 1 1-kb blunt-ended *Xho*I-*Sal*I fragment of plasmid pMClneo (ref 13) inserted in the blunt-ended *Eco*RI site of exon II of the 10-kb *Bam*HI fragment of the *β2-m* gene The *neo* gene in this construct is driven by the *tk* promoter with an upstream tandem repeat of the polyoma mutant enhancer region[13] Small bars indicate the position of the primers used for PCR A box indicates the position of the 500-base pair (bp) *Bam*HI-*Bgl*I (*β2-m)* fragment used as hybridization probes in the PCR and/or Southern Blot analyses The 900-bp *Eco*RI-*Sal*I fragment used as a *neo* probe was derived from plasmid pMClneo (ref 13) Roman numerals denote the exons of the *β2-m* gene Arrows indicate the sizes of *Bgl*I, *Hin*dIII and *Eco*RI fragments hybridizing with the *β2-m* probe in DNA of parental D3 cells (top) and the predicted sizes in a targeted *β2-m* gene (bottom) Bg* is a *Bgl*I polymorphism present in exon II of the targeting vector (C57BL/6 DNA-derived, *β2-m*^{b} allele), but absent in the ES cell DNA (129J mouse-derived, *β2-m*^{a} allele) B, *Bam*HI site, Bg, *Bgl*I site, E, *Eco*RI site, H, *Hin*dIII site *b*, Southern-blot analyses of the structure of the *β2-m* locus in the DNA of parental D3 cells and two targeted clones A and B Hybridization probes as in *a* Triangles indicate diagnostic restriction enzyme fragments obtained after *Bgl*I, *Hin*dIII and *Eco*RI digestion Bars indicate migration of *Hin*dIII-digested λ DNA markers DNA (10 μg) was digested with the indicated enzymes, run on a 0 7% agarose gel, and blot-hybridized by standard procedures

TABLE 2 Germ-line transmission of ES cell genome

imals from injected blastocysts

Injected ES cells	No blastocysts injected*	Progeny Born	Progeny Survived	Chimaeric mice Total tested†	Chimaeric mice Female	Chimaeric mice Male
Parental D3	18	4	4	4	2	2
Clone A	49	7	3	2	0	2
Clone B	40	17	8	8	0	8

ffspring of male chimaeras

Chimaera derived from	Mouse no	Extent of coat-colour chimaerism†	No of litters	No of germ-line offspring with ES genome/per total‡ (% transmission)
Parental D3	1	90%	5	21/33 (64%)
	2	70%	3	17/28 (61%)
Clone A	A1	5%	7	0/40 (0%)
Clone B	B1	80%	5	1/53 (2%)
	B2	95%	3	16/16 (100%)
	B3	90%	1	7/7 (100%)
	B4	50%	Sterile	
	B5	95%	5	22/22 (100%)
	B6	90%	4	5/23 (22%)
	B7	50%	3	4/19 (21%)

* ES cells (10–20) were injected into C57BL/6J blastocysts and transferred to uteri of pseudopregnant recipients

† Assessed by the contribution of the agouti coat colour contributed by D3 cells (129J, agouti) on the black background of C57BL/6J mice

‡ Assessed by the presence of the dominant agouti coat colour in offspring after breeding with C57BL/6J females

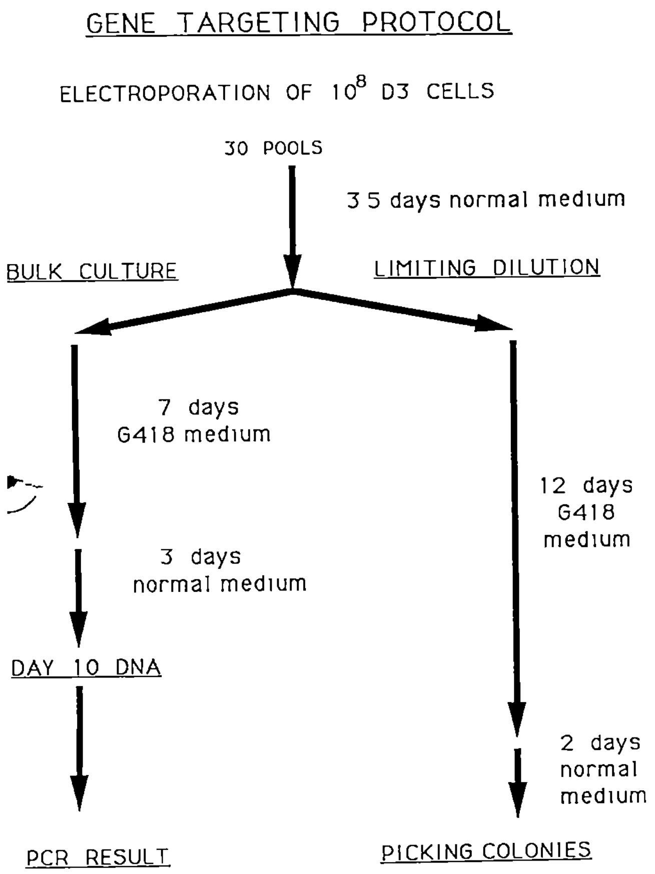

FIG 2 Electroporation of ES cells D3 ES cells[12] were routinely grown on γ-irradiated (2,000 rad) primary embryonic fibroblasts (EF) in DMEM medium supplemented with 15% FCS, 1×non-essential amino acids (Gibco) and 10^{-4} M β-mercaptoethanol Electroporation of 1×10^8 D3 cells using a BTX 300 transfector (50 μF, 275 V) with 25 μg ml^{-1} linearized vector DNA was performed as previously described[13] The electroporation conditions result in an 80% plating efficiency in comparison with non-electroporated D3 cells Electroporated cells were divided into 30 independent pools and seeded into individual 25-cm^2 tissue culture flasks on a feeder layer of EF Selection procedure After three one-half-day's growth in normal medium, cells of individual pools were trypsinized and divided into two halves One half was seeded into a 48-well plate on irradiated EF derived from transgenic mice expressing a *tk-neo* construct[12] and selected for 12 days in medium containing 150 μg ml^{-1} (active substance) G418 From day 6–14, the medium was supplemented with 15% D3 cell-conditioned medium (0 22-μm filtered) and 1,000 U ml^{-1} recombinant mouse leukaemia inhibitory factor (LIF)[24] The other half was seeded as a bulk culture in a 25-cm^2 flask on the irradiated *neo*-expressing EF The bulk cultures were selected for 7 days in medium containing 150 μg ml^{-1} G418 and subsequently for 3 days in normal medium Thereafter, DNA derived from the bulk cultures were tested by PCR for the presence of gene-targeted clones Subsequently, the sibling cultures in the 48-well dishes were used to isolate clones from those pools scored positive by PCR Clones were expanded and screened by PCR Thereafter, DNA of PCR^+ clones was characterized by Southern-blot analyses as outlined in Fig 1 For PCR, DNA from bulk cultures or individual clones was isolated, treated with proteinase K, deproteinized with phenol–chloroform, precipitated and washed with ethanol and dissolved in 10 mM Tris buffer pH 8 0, 1 mM EDTA by standard procedures DNA (250 ng) was tested by PCR under conditions recommended by the supplier (Perkin Elmer Cetus) with the addition of 2 mM $MgCl_2$, in 50 μl containing 200 pmol of each primer (*neo*, ATATTGCTGAAGAGCTTGGCGGCGAATGGG, *β2-m*, AAGGGAGGGAGAGAAGGAGAAGGTTAGCC) PCR was run for 40 cycles using a thermal cycler (Perkin Elmer Cetus) Denaturation was performed for 1 5 min at 94 °C, annealing for 2 min at 63 °C and extension for 4 min at 72 °C A 25-μl reaction sample was run on an 0 8% agarose gel and blot-hybridized by standard procedures

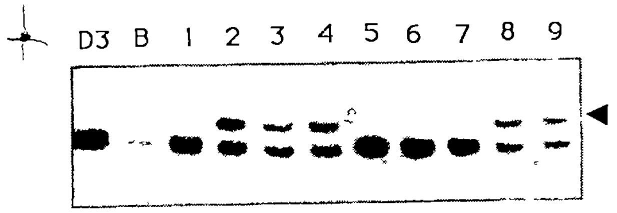

FIG 3 Germ-line transmission of mutant *b2-m* gene Southern blot of DNA from offspring of chimaeric males B2 and B3 (Table 2) The DNA was digested with *Eco*RI and probed with a *β2-m* probe as described in Fig 1 The arrowhead marks the fragment from the mutant *β2-m* gene (6 4 kb, see Fig 1) D3, parental ES line, clone B, mutant clone injected into blastocysts, numbers refer to individual offspring

pool was seeded into 48-well plates and propagated for 12 days in the presence of G418. As soon as pools containing targeted cells were identified by PCR analysis of the bulk cultures, clones were isolated from the corresponding sibling 48-well cultures, expanded and screened by PCR. The successful identification of targeted clones by this screening protocol depends critically on the plating efficiency of the trypsinized cells at day 3.5 after the initial electroporation. The use of a low trypsin concentration (0.025%)[19] and the seeding of the cells at high density ($2\text{–}4\times10^5$ cells cm^{-2}) resulted in a plating efficiency of >50%, thereby permitting partition of daughters of a single initially targeted cell into both sibling pools.

The results of two independent experiments are summarized in Table 1. In the first experiment, 7/32 cell pools, and in the second experiment, 19/28 cell pools, were identified to contain targeted cells by PCR. A large fraction of clones present in each of a total of seven positive pools were isolated, and targeted clones were identified by the PCR reaction in each pool. To confirm that the *β2-m* gene in the targeted clones was disrupted, DNA from seven independent clones was characterized by Southern-blot analysis using probes specific for the *β2-m* and neo^r genes (Fig. 1*b*). DNA of six clones showed the additional diagnostic *Bgl*I, *Eco*RI and *Hin*dIII fragments predicted from disruption of the *β2-m* gene by the targeting vector (Fig. 1*a*). DNA of one clone contained the diagnostic *Eco*RI and *Hin*dIII fragments, but lacked the *Bgl*I site in exon II (data not shown), most probably caused by a small mutation or deletion which can occur during a homologous recombination event[15].

The targeting frequency in our experiments was ~1 in 2.5×10^6 electroporated input cells or 1/25 of the G418^r clones. This is one to three orders of magnitude higher than the targeting frequencies reported for other genes[13-16,25]. There are at least two factors that could contribute to the high efficiency of *β2-m*-gene targeting. The gene could contain a hotspot of recombination, as suggested by the clustering of rearrangements in the first intron in the DNA of independent *β2-m* gene variants in lymphoma cell lines[20]. In addition, the lack of a polyadenylation site in the vector reduced the number of drug-resistant clones from non-homologous integration events by about fourfold (data not shown).

Northern-blot analysis indicated the presence of little, if any, *β2-m*-specific messenger RNA in undifferentiated D3 cells (data not shown). Therefore, high-frequency homologous recombination in ES cells is clearly not dependent on a high steady-state mRNA level as exemplified in the study described here for the *β2-m* gene, and observed previously for other genes[16,25]. In our view, the chromatin structure could be more important. It has been shown, for example, that the *β2-m* gene in F9 cells, which express only trace amounts of *β2-m* mRNA, is much more susceptible to digestion by DNAase I than it is in differentiated F9 cells, which express moderate levels of *β2-m* mRNA[21]. This suggests that the gene, even if it is expressed a little or not at all is in an 'opened' chromatin conformation.

To derive animals carrying a disrupted *β2-m* gene, two different targeted clones were injected into C57BL/6J host blastocysts. Whereas clone A resulted in a few animals with a low degree of chimaerism, extensive and healthy chimaeras were derived from clone B (Table 2). Chimaeric males were bred with C57BL/6J females. So far, three males have transmitted the ES cell genome to all of their offspring and three other males have transmitted it to part of their offspring (Table 2). A 100% transmission is expected for 'pseudomales', which are derived from a female host blastocyst injected with the male ES cell line[22]. In such animals, the XX germ cells of the female host are not capable of forming functional spermatozoa. So far, Southern-blot analysis was performed with tail DNA of 17 offspring from pseudomales B2 and B3 (Table 2), and revealed the presence of the additional *Eco*RI fragment predicted by inheritance of the disrupted *β2-m* gene in eight animals (Fig. 3). Our results, therefore, indicate that the disrupted *β2-m* gene is genetically transmitted according to mendelian expectations.

The animals heterozygous for the *β2-m*-gene disruption have so far not shown any detectable phenotype and will be intercrossed to obtain animals homozygous for the mutation. We expect that homozygous animals will lack MHC class I molecules on their cell surface and, as a consequence, be severely immunocompromised. Considering the various other functions the *β2-m* protein could have, failure to express the gene may lead to disturbance of embryonic development. The *β2-m* gene is expressed as soon as the two-cell stage[23], and thus is one of the earliest genes to be activated during mammalian development that is known. The phenotype of homozygous mutant mice should therefore help in understanding the various functions that the *β2-m* protein, as well as its ligands, has in the life cycle of mammals. □

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1. Klein J., Juretic A., Baxevanis C. N. & Nagy Z. A. *Nature* **291**, 455–460 (1981).
2. Zinkernagel R. M. & Doherty P. C. *Adv. Immun.* **27**, 51–117 (1979).
3. Fehlmann M. *et al. Proc. natn. Acad. Sci. U.S.A.* **82**, 8634–8637 (1985).
4. Due C., Simonsen M. & Olsson L. *Proc. natn. Acad. Sci. U.S.A.* **83**, 6007–6011 (1986).
5. Schreiber A. B., Schlessinger J. & Edidin, M. *J. cell Biol.* **98**, 725–731 (1984).
6. Solano A. R. *et al. Proc. natn. Acad. Sci. U.S.A.* **85**, 5087–5091 (1988).
7. Singh P. B., Brown R. E. & Roser B. *Nature* **327**, 161–164 (1987).
8. Yamazaki K. *et al. Science* **240**, 1331–1332 (1988).
9. Simister N. E. & Mostov K. E. *Nature* **337**, 184–187 (1989).
10. Brinckerhoff C. E., Mitchell T. I., Karmilowicz, M. J., Kluve-Beckerman, B. & Benson M. D. *Science* **243**, 655–657 (1989).
11. Robertson, E., Bradley A., Kuehn M. & Evans M. *Nature* **323**, 445–448 (1986).
12. Gossler A., Doetschman T., Korn R., Serfling, E. & Kemler R. *Proc. natn. Acad. Sci. U.S.A.* **83**, 9065–9069 (1986).
13. Thomas K. R. & Capecchi M. R. *Cell* **51**, 503–512 (1987).
14. Mansour S. L., Thomas K. R. & Capecchi M. R. *Nature* **336**, 348–352 (1988).
15. Doetschman T., Maeda, N. & Smithies O. *Proc. natn. Acad. Sci. U.S.A.* **85**, 8583–8587 (1988).
16. Joyner A. L., Skarnes W. C. & Rossant, J. *Nature* **338**, 153–156 (1989).
17. Zimmer A. & Gruss P. *Nature* **338**, 150–153 (1989).
18. Thompson, S., Clarke A. R., Pow A. M., Hooper M. L. & Melton, D. W. *Cell* **56**, 313–321 (1989).
19. Rudnicki M. A. & McBurney M. W. in *Teratocarcinomas and Embryonic Stem Cells* (ed. Robertson, E. J.) 19–49 (IRL, Oxford 1987).
20. Potter T. A., Frankel W., Zeff R. A. & Rajan, T. V. *J. Immun.* **138**, 1270–1274 (1987).
21. Croce C. M. *et al. Proc. natn. Acad. Sci. U.S.A.* **78**, 5754–5758 (1981).
22. Bradley A., Evans M., Kaufman M. & Roberston E. *Nature* **309**, 255–256 (1984).
23. Sawicki J. A., Magnuson T. & Epstein C. J. *Nature* **294**, 450–451 (1981).
24. William R. L. *et al. Nature* **336**, 684–587 (1988).
25. Johnson R. *et al. Science* **245**, 1234–1236 (1989).

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Developmental regulation of stage-specific ribosome populations in *Plasmodium*

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THE *Plasmodium* parasites are so far unique in biology in possessing developmentally regulated ribosomal RNA gene units[1]. Two different genes encode their small subunit rRNAs: one gene (*A*) yields transcripts predominant in the asexual blood-stage parasites, and the other (*C*) is mainly transcribed in the sporozoite forms that develop in the mosquito[1,2]. Developmental control of events allowing a switch in the complement of ribosomes must coordinate the production of the new class with selective inactivation and removal of the old. We show here that in *P. falciparum* the switch from *A* to *C* gene expression involves the control of

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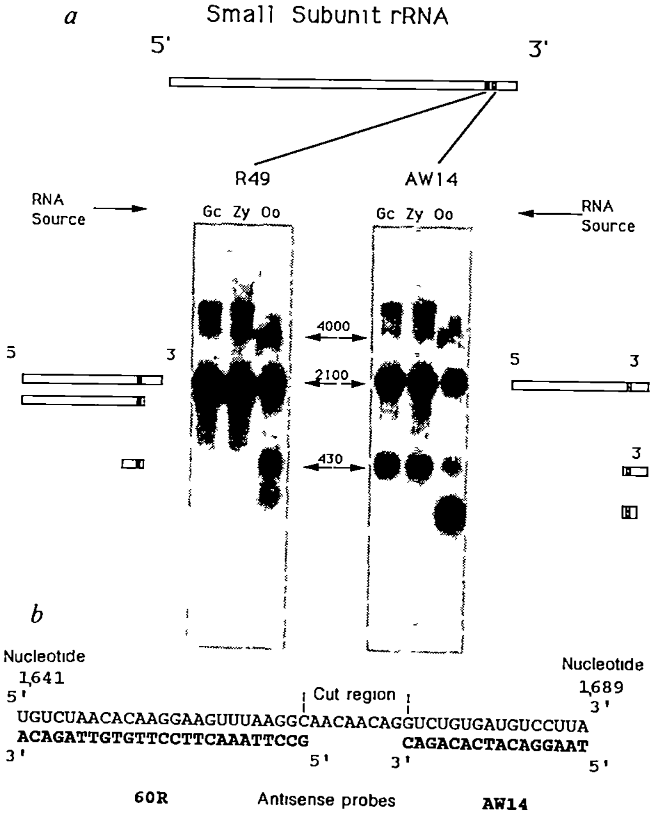

FIG 3 *a*, The *A*-gene small-subunit rRNA transcript is selectively cleaved during sexual development The probes were complementary to the regions indicated AW14 has the sequence 5'-TAAGGACATCACAGAC-3' and is complementary to nucleotides 1,674–1,689, probe 49R has been described[9] and is complementary to nucleotides 1,606–1,634, probe 60R has the sequence 5'-GCCTTAAACTTCCTTGTGTTAGACA-3' and was used to map the precise location of the initial cut Methods are described in the legend to Fig 2 *b*, Site of the initial cut in the *P falciparum A* gene The sequence of the *A*-gene small-subunit rRNA region between nucleotides 1,641–1,689 is given at the top The two antisense oligonucleotide probes, AW14 and 60R, are shown in bold at the appropriate locations The two probes hybridize at stringency to the two different processed RNA fragments in gametes and gametocytes The region of the first cut site is thus located to a region of no more than 10 nucleotides between the two probes

The structure of the stable precursor in gametocytes from the ribosomal *C* gene is also significant to the understanding of the transition process Oligonucleotide mapping has demonstrated that the entire 1,800 extra nucleotides in the precursor lies 5' to the mature transcript (data not shown) Such a precursor structure is like that of small-subunit rRNA precursor molecules found in yeast mutants that lack the gene for a ubiquinated ribosomal protein[6] It is possibile that a stage- (and binding sequence-) specific ubiquinated ribosomal protein is produced to aid the maturation of each type of rRNA transcript, this is under investigation at present

Although much is known about the generation of mature rRNA transcripts from the single multigenic precursor molecule, our results demonstrate that it can be a regulated, developmentally controlled process This control is minimally exerted at three levels, transcription, RNA processing and RNA degradation, facilitating the novel phenomenon of ribosome biogenesis, switching and turnover in *Plasmodium* Specific factors involved in these events during the hepatic stage of the *Plasmodium* life cycle could prove to be targets for novel drugs directed against malaria parasites and other micro-organisms These events can be easily assayed *in vitro* to test such drugs It is possible that other organisms with a defined dimorphic lifestyle could also display this type of control ☐

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1 Gunderson J H *et al Science* **238**, 933–937 (1987)
2 McCutchan T F *et al Molec Biochem Parasitol* **28**, 63–68 (1988)
3 Murgola E J Hijazi K A Goringer H U & Dahlberg A E *Proc natn Acad Sci USA* 8 4162–4165 (1988)
4 Dahlberg A E *Cell* **57**, 525–529 (1989)
5 Dams E *et al Nucleic Acids Res* **16**, r87–r173 (1988)
6 Finley D Bartel B & Varshavsky A *Nature* **338**, 394–401 (1989)
7 Carter R Graves P M Quakyi I A & Good M F *J Exp Med* **169**, 135–147 (1989)
8 Maniatis T Fritsch E F & Sambrook J *Molecular Cloning A Laboratory Manual* 194–195 (Col Spring Harbour Laboratory New York 1982)
9 Waters A P & McCutchan T F *Lancet* **i**, 1343–1346 (1989)

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Molecular cloning and expression of human hepatocyte growth factor

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HEPATOCYTE growth factor (HGF) is the most potent mitogen for mature parenchymal hepatocytes in primary culture, and seems to be a hepatotrophic factor that acts as a trigger for liver regeneration after partial hepatectomy and liver injury. The partial purification and characterization of HGF have been reported[1–4]. We have demonstrated that pure HGF from rat platelets is a new growth factor[5,6] effective at concentrations as low as 1 ng ml^{-1}.

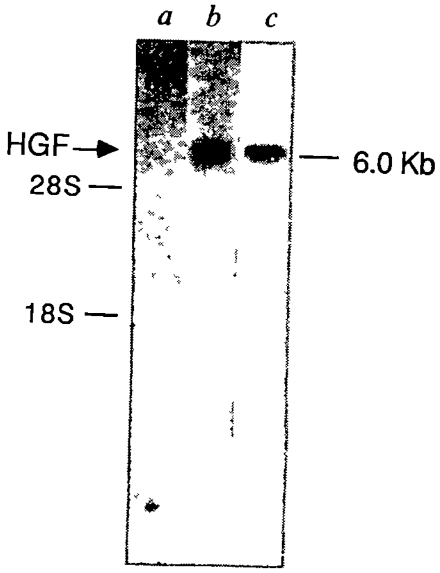
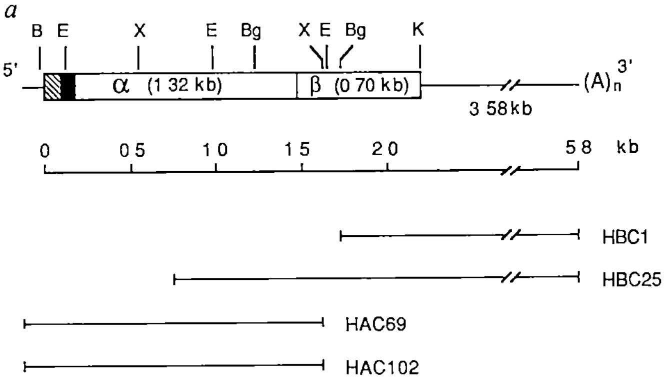


FIG 1 Northern hybridization of mRNAs from rat and human livers Poly(A)$^+$ RNAs were isolated from livers of normal adult rat (lane *a*) and CCl$_4$-treated rat (lane *b*) Lane *c* is human liver poly(A)$^+$ RNA (Clontech) The positions of the 28S and 18S ribosomal RNA markers are shown on the left Blots were hybridized with rat HGF cDNA (clone RBC1 1 4 kb insert)
METHODS Poly(A)$^+$ RNAs (1 μg per lane) were electrophoresed into a 1% agarose gel containing 0 66 M formaldehyde[17] and blotted onto a nylon membrane filter (Genescreen plus, NEN) The filter was hybridized in 5× SSPE (800 mM NaCl, 50 mM sodium phosphate, 5 mM EDTA, pH 7 8), 5× Denhardt's solution, 10% dextran sulphate, 40% formamide, 0 1% SDS, 1 mM sodium pyrophosphate, 0 1 mg ml^{-1} sonicated *E coli* DNA at 40 °C overnight with a ^{32}P-labelled probe prepared from the 1 4 kb *Eco*RI fragment of the cDNA clone RBC1 (Amersham multiprime DNA labelling system) The filter was washed with 1 ×SSC containing 0 1% SDS at 60 °C, dried and then exposed on X-ray film for 2 days at −80 °C using an intensifying screen

he effects of HGF and epidermal growth factor (EGF) are dditive. The activity of HGF is not species-specific, although it loes not stimulate growth in Swiss 3T3 fibroblasts[5]. HGF has a elative molecular mass (M_r) of 82,000 and is a heterodimer omposed of a large α-subunit of M_r 69,000 and a small β-subunit f M_r 34,000 (ref. 6). Here we report the amino-acid sequence of uman HGF determined by complementary DNA cloning and the xpression of biologically active human HGF from COS-1 cells ransfected with cloned cDNA. The nucleotide sequence of the uman HGF cDNA reveals that both α- and β-chains are conained in a single open reading frame coding for a pre-pro precursor rotein of 728 amino acids.

HGF was purified to homogeneity from the platelets of 3,000 ats by a four-step procedure[5,6]. The yield was about 60 μg,



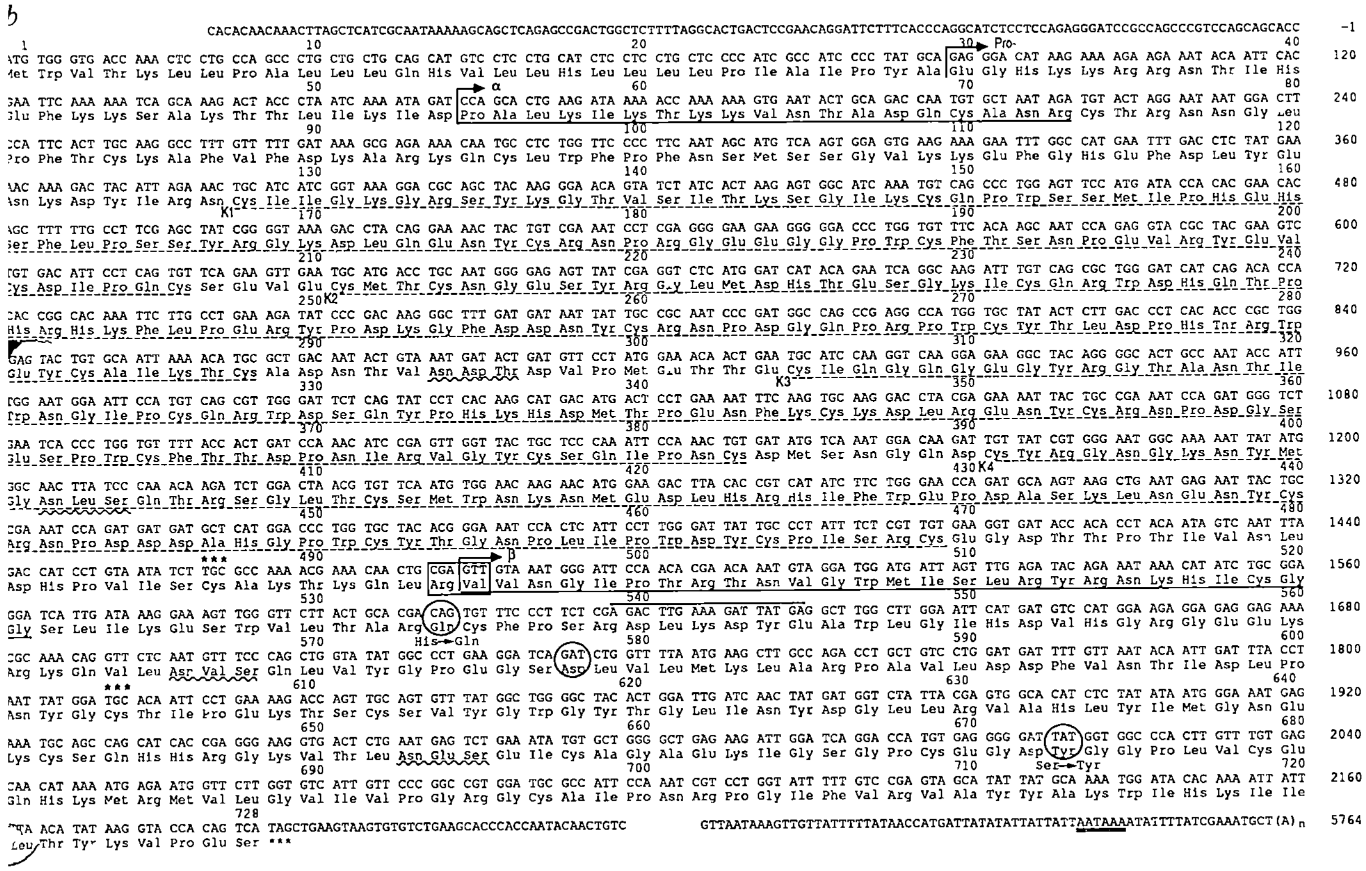


FIG. 2 The restriction map, nucleotide sequence and deduced amino-acid sequence of human HGF cDNA. *a*, Untranslated sequences are represented by a line and coding sequences are boxed. The open portion indicates the coding regions for the α- and β-chains. Hatched and black areas show the coding region for the signal peptide and pro-sequences, respectively. The scale is in nucleotides from the initiation codon ATG. *b*, The nucleotide numbers are given on the right, and amino acid numbers are given throughout. Experimentally determined N-terminal sequences of HGF α- and β-chains are underlined. The proteolytic cleavage site is boxed. An interchain S–S bridge is indicated by asterisks. Dotted underlines indicate the kringle domains. The primer for the construction of the cDNA library is overlined. The four potential N-glycosylation sites are marked with wavy lines. In the β-chain of HGF, amino acid numbers 534 (Gln) and 673 (Tyr) correspond to the histidine and the serine in plasmin, respectively, which form the active centre for protease action.

METHODS. A long oligodeoxynucleotide probe encoding the N-terminal amino-acid sequence (1–15) of the rat HGF β-chain was synthesized on an Applied Biosystems Model 381A DNA synthesizer. This probe (44-mer) containing inosine is 3'>CAICAITTICCITAIGGITGIGTITGITGICAIC-CIACCTACCA<5'. These oligodeoxynucleotides were labelled by phosphorylation using T4 polynucleotide kinase and [γ-^{32}P]ATP (ref. 18). Rat liver double-stranded cDNA was synthesized from liver poly(A)$^+$ RNA that had been isolated from rats 10 h after they had been injected with CCl_4. A cDNA synthesis system (Amersham UK) with oligo dT primer was used and the cDNA was methylated by *Eco*RI methylase and then ligated into an *Eco*RI linker[19]. After *Eco*RI digestion, the cDNAs were ligated into *Eco*RI-digested λgt10 vector (Stratagene) and packaged *in vitro* using Gigapack Gold (Stratagene)[20]. The recombinant phages were plated on *E. coli* NM514 and a replica nitrocellulose filter prepared[21]. From 2 μg poly(A)$^+$ RNA, 2×10^6 transformants were obtained. These clones were screened by hybridization to a probe 44 bases long and containing inosine. Filters were hybridized with the ^{32}P-end-labelled 44-mer probe for 12 h at 45 °C in 5× SSC, 5× Denhardt's solution, 0.1% SDS and 100 μg ml^{-1} denatured salmon testis DNA. The filters were then washed at 50 °C in 5×SSC containing 0.1% SDS, air-dried, and autoradiographed using Fuji X-ray film. We obtained one positive clone containing the 1.4 kb *Eco*RI fragment, designated as RBC1. The human liver cDNA library was constructed from human liver poly(A)$^+$ RNA (Clontech) as before, except that cDNAs larger than 4 kb were fractionated in a 1% agarose gel, pooled, and then ligated. Recombinants (2×10^5) were screened with the ^{32}P-labelled 1.4 kb *Eco*RI fragment of RBC1 (Amersham multiprime DNA labelling system). Two clones, HBC1 and HBC25, were obtained. The cDNA library was constructed using 5'-TCATAATCTTTCAAGTCT as a primer, 6×10^5 recombinants were screened using the 0.7 kb *Eco*RI fragment prepared from HBC25 to yield 100 positive clones and the two longest clones, HAC69 and HAC102, were isolated. The cDNA inserts of the clones HAC69 and HBC25 were subcloned into M13mp18, M13mp19 and pBluescript (Stratagene) and sequenced by the dideoxy-termination method[22] using Sequenase (US Biochemicals).

corresponding to a recovery of 23% The purified HGF was reduced, carboxymethylated and fractionated by reverse-phase HPLC on a C_4-column to isolate the α- and β-chains We determined the N-terminal sequence (one-letter code) of the α- and β-chains as PLVKIKTKKVNSADECANR and VVNGIPTQTTVGWMVSLKYRNKHICGG, respectively

We employed the following strategy to isolate a cDNA encoding complete human HGF First, we cloned rat HGF cDNA using a hybridization probe designed to have a sequence corresponding to the N-terminus of the rat HGF β-chain, and then isolated human HGF cDNA using the rat HGF cDNA as probe We investigated the tissue distribution of HGF in rats to establish a good source of HGF messenger RNA and found that the livers of rats treated with CCl_4 contain large amounts of HGF To clone HGF cDNA, a cDNA library constructed in λgt10 from poly$(A)^+$ RNA extracted from the livers of CCl_4-treated rats was screened with a single 44-mer oligodeoxynucleotide whose sequence corresponded to the first 15 amino acids of the β-chain Screening 2×10^6 clones gave one positive clone with a 1 4-kilobase (kb) insert (clone RBC1) Nucleotide sequencing of this insert revealed an open reading frame predicting the translation of a 404-residue protein and a 3′-untranslated sequence (data not shown) We confirmed that the 27 N-terminal amino acids of the β-chain were included in the 233 amino acids of the rat HGF β-chain encoded by the 1 4 kb insert

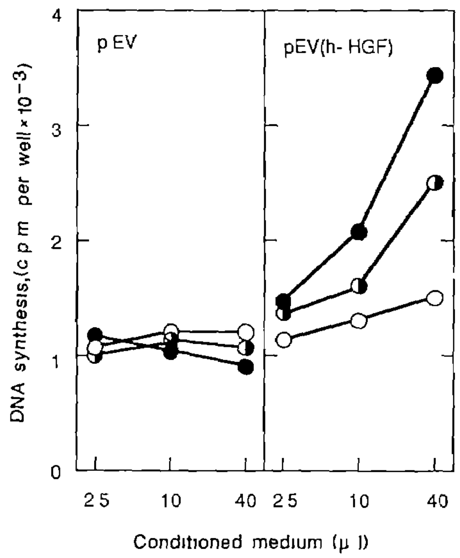


FIG 3 Expression of the cloned cDNA encoding the human HGF in COS-1 monkey kidney cells HGF activity in the conditioned medium from cultures of COS-1 cells transfected with HGF cDNA (right) and the corresponding vector without insert (left) O, 1-day culture, ◑, 2-day culture, ●, 3-day culture

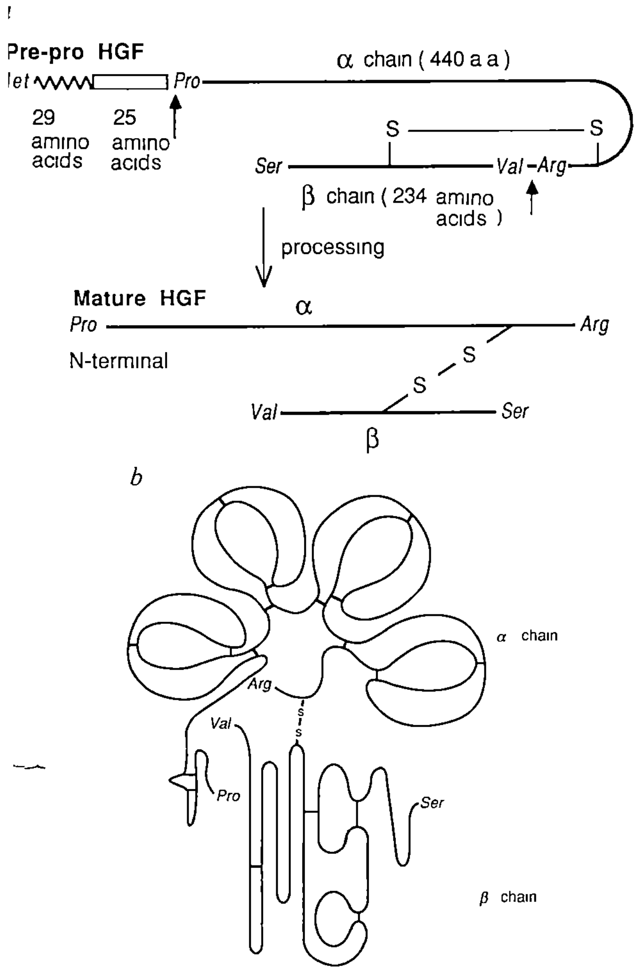
METHODS The vector pEV contains the SV40 early promoter, polyadenylation sequence and the origin of replication The human HGF cDNA (*BamHI-PstI* 2 95 kb fragment) was inserted into pEV vector downstream of the SV40 early promoter The constructed plasmid DNAs (pEV) were introduced to COS-1 cells by the DEAE-Dextran method[23] COS-1 cells were seeded at a density of 2×10^5 cells into 60 mm tissue culture plates two days before transfection After washing the plates with 20 mM Tris-HCl (pH 7 4) containing 150 mM NaCl (TBS), 10 µg plasmid DNA in TBS (600 µl) containing 500 µg ml^{-1} DEAE-dextran was used for transfection for 30 min at room temperature The transfected cells were incubated with Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS) and 100 µM chloroquine for 3 h at 37 °C, followed by treatment with DMEM containing 10% FCS and 10% dimethylsulphoxide for 2 5 min at room temperature This medium was replaced with DMEM containing 10% FCS and 50 µg ml^{-1} gentamicin and cultured for 3 days at 37 °C The culture medium was collected at 24, 48 and 72 h after transfection, and HGF activity assayed as described[24,25] Aliquots of conditioned medium (2 5, 10 or 40 µl) were added to the adult rat hepatocyte cultures (1 6 cm diameter well 0 5 ml medium) 20 h after plating, and 18 h later, ^{125}I-labelled deoxyuridine (0 3 µCi ml^{-1}) was added Six hours later, DNA synthesis was assayed by measuring the incorporation of ^{125}I-labelled deoxyuridine A purified preparation of rat HGF from platelets (2ng ml^{-1}) and saline used as controls gave 5,400 c p m and 1,200 c p m incorporation, respectively

Northern hybridization using the RBC1 clone showed that levels of rat HGF mRNA were negligible in normal liver but increased markedly in the injured livers of CCl_4-treated rats, and that the mRNA was ~6 kb in size We next investigated whether we could use human liver mRNA as a source of mRNA for human HGF cDNA cloning HGF mRNA was clearly detectable in human liver and was the same size as the rat HGF mRNA, as shown in Fig 1 A human liver cDNA library was therefore constructed in λgt10, using oligo(dT) primer The screening of 2×10^5 cDNA clones with ^{32}P-labelled RBC1 gave two positive clones (HBC1 and HBC25) and two additional cDNA clones (HAC69 and HAC102) were obtained from the unique 18-mer primer-extended cDNA library Four clones were characterized in detail and are illustrated in Fig 2*a*, together they span 6 kb which is sufficient to encode the entire mRNA that we detected by northern blotting The sequence comprises a 5′ non-coding region of 134 nucleotides, a single open reading frame of 2,184 nucleotides and 3,580 nucleotides of the 3′ non-coding region, which contains an A^+U-rich sequence (Fig 2*b*)

A polypeptide of 728 amino acids is encoded by human HGF cDNA in a single open reading frame which includes both the α- and β-chains The calculated relative molecular masses (M_r) of the human HGF pre-precursor and the mature form are 83,126 and 76,879 respectively The N-terminal amino acid of the α-chain is preceded by 54 amino acids, starting with a methionine Of these 54 amino acids, most of the first 29 are hydrophobic, which is typical of a signal sequence[7] The next 25 (amino acids 30-54) preceding the N-terminus of the α-chain constitute the pro-sequence The α-chain runs from amino acids 55 to 494, and the β-chain from 495 to 728 From the known substrate specificity of proteases, this Gln-Leu-Arg-Val sequence should be accessible to a trypsin-like protease such as plasmin or plasma kallikrein[8] After biosynthesis, the chains are separated by a trypsin-like protease at an Arg-Val site (Fig 4*a*) The predicted M_r of the α-chain, which consists of 440 amino acids, is 50,808, and for the β-chain, which consists of 234 amino acids, it is 26,089 The HGF contains four putative asparagine-linked glycosylation sites, which are located at positions 294 and 402 of the α-chain and at positions 566 and 653 of the β-chain The difference between the M_rs of the HGF subunits as determined by SDS-PAGE and those predicted from the cDNA sequence may be due to carbohydrate residues, which have been demonstrated by binding to concanavalin A-Sepharose columns (data not shown)

We demonstrated that biologically active HGF could be expressed from its cDNA in transient expression experiments in COS-1 cells As shown in Fig 3, the conditioned medium from the cells transfected with the plasmid pEV(hHGF) revealed the dose-dependent stimulation of DNA synthesis of adult rat hepatocytes in primary culture This activity was abolished by heating at 100 °C for two minutes and we found that it was additive with insulin and EGF, which suggested to us that it was attributable to HGF

We found considerable homology (38%) between the overall amino-acid sequence of the pro-HGF precursor and plasminogen[9], but neither plasminogen nor plasmin has HGF activity (data not shown) Plasminogen has 791 amino acids and contains five kringle structures, the α-chain of HGF would contain four kringle structures Prothrombin, tissue plasminogen activator, urokinase and coagulation factor XII also contain a kringle structure[10] Although the kringle domain is thought to play a part in protein-protein interaction, its physiological function is unknown On the other hand, the mature β-chain has extensive homology (37%) with a serine protease domain of plasminogen, but the histidine and serine residues of the protease active site are replaced in the HGF β-chain by glutamine and tyrosine, respectively, so HGF should have no proteolytic



IG 4 Structural features of human HGF *a*, Schematic representation of ie structure and processing of pre-pro HGF The arrows indicate the rocessing sites The wavy line and unfilled line represent the signal peptide nd pro-sequences, respectively *b*, Predicted primary structure of human GF An interchain S-S bridge is formed between Cys 487 in the α-chain nd Cys 604 in the β-chain

ctivity Other examples of amino acid replacements in the serine rotease active site include haptoglobin[11] and protein Z[12], which ave no proteolytic activity In mature HGF, Cys 604 in the β-chain and Cys 487 in the α-chain seem to form an interchain ridge in a position comparable to that of the interchain bridge ound in plasmin The predicted structure of human HGF is hown in Fig 4*b*

A marked increase in HGF-like activity has been reported in he sera of both partially hepatectomized[1 2,13] and hepatotoxin-reated rats[14 15] and of patients with fulminant hepatic failure[16] Ve find a time-dependent increase in HGF activity in rat liver fter administration of CCl_4, which reaches a maximum of about 0 times the initial level at 36 hours The level of HGF mRNA s negligible in normal rat liver, but increases dramatically in njured liver 10 hours after the administration of CCl_4 We have lemonstrated that rat liver-derived HGF has the same chemical nd biological properties as rat platelet-derived HGF (manucript in preparation) These findings indicate that the liver itself s producing HGF HGF in plasma from patients with fulminant iepatic failure is probably derived from the injured liver as uman platelets do not contain a significant amount of HGF, nlike rat platelets Thus HGF seems to be an important growth actor in the stimulation of liver regeneration after partial iepatectomy and liver injury

The availability of functionally active recombinant human GF will enable us to study the HGF receptor on hepatocytes nd the role of HGF during liver regeneration *in vivo* HGF may eventually be useful clinically for enhancing liver regeneration after surgery and in the diagnosis and treatment of hepatitis ☐

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1 Nakamura T Nawa K & Ichihara A *Biochem biophys Res Commun* **122,** 1450-1459 (1984)
2 Thaler F J & Michalopoulos G K *Cancer Res* **45,** 2545-2549 (1985)
3 Ghoda E *et al J clin Invest* **81,** 414-419 (1988)
4 Rusell W E McGowan J A & Bucher N R L *J Cell Physiol* **119,** 193-197 (1984)
5 Nakamura T Teramoto H & Ichihara A *Proc natn Acad Sci USA* **83,** 6489-6493 (1986)
6 Nakamura T Nawa K Ichihara A Kaise N & Nishino T *FEBS Lett* **224,** 311-316 (1987)
7 Von Heijine G *Eur J Biochem* **133,** 17-21 (1983)
8 Kawabata S *et al Eur J Biochem* **172,** 17-25 (1988)
9 Sottrup-Jensen L Claeys H Zajdel M Petersen T E & Magnusson S *Progress in Chemical Fibrinolysis and Thrombolysis* Vol 3 191-209 (Raven New York 1978)
10 Furie B & Furie B C *Cell* **53,** 505-518 (1988)
11 Kurosky A *et al Proc natn Acad Sci USA* **77,** 3388-3392 (1980)
12 Højrup P Jensen M S & Petersen T E *FEBS Lett* **184,** 333-338 (1985)
13 Morley C G D & Kingdom H S *Biochim biophys Acta* **308,** 260-275 (1973)
14 Morley C G D & Boyer J L *Biochim biophys Acta* **477,** 165-176 (1977)
15 Ando T *et al Hepatology* (in the press)
16 Nakayama, H *et al Biomed Res* **6,** 231-237 (1985)
17 Maniatis T Fritsch E F & Sambrook J *Molecular Cloning A Laboratory Manual* 202-203 (Cold Spring Harbor Laboratory New York 1982)
18 Maniatis T Fritsch E F & Sambrook J *Molecular Cloning A Laboratory Manual* 125-126 (Cold Spring Harbor Laboratory New York 1982)
19 Gubler U & Hoffman B J *Gene* **25,** 263-269 (1983)
20 Huynh T V Young R A & Davis R W *DNA Cloning* Vol 1 49-78 (IRL Oxford 1985)
21 Benton W D & Davis R W *Science* **196,** 180-182 (1977)
22 Sanger F Miklen S & Coulson A R *Proc natn Acad Sci USA* **74,** 5463-5467 (1977)
23 Berger S L & Kimmel A R *Meth Enzym* **102,** 684-704 (Academic London 1987)
24 Nakamura T Tomita Y & Ichihara A *J Biochem* **94,** 1029-1035 (1983)
25 Nakamura T Arakaki R & Ichihara A *Expl Cell Res* **179,** 488-497 (1988)

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Anti-HLA-A2 antibody-enhancement of peptide association with HLA-A2 as detected by cytotoxic T lymphocytes

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MOST cytotoxic T lymphocytes (CTL) not only recognize epitopes of viral or other foreign proteins in association with class I major histocompatibility complex (MHC) molecules, but also recognize target cells sensitized with short synthetic peptides representing the epitopes[1] There is increasing evidence that these synthetic peptides associate with the class I molecule both at the cell surface and intracellularly[2] We have now investigated the effect of a monoclonal antibody specific for HLA-A2 and HLA-B17 (B57/58) molecules (antibody MA2 1)[3] on the sensitization of target cells with peptide for lysis by HLA-A2-restricted CTL Previously, anti-HLA class I monoclonal antibodies have been shown to inhibit the recognition of target cells, infected with influenza A virus, by virus-specific CTL[4,5] We find, however, that target cells treated with MA2.1 antibody can be sensitized with peptide for CTL lysis much more rapidly than untreated cells, or at >100-fold lower peptide concentration than that required for sensitization of untreated cells This implies that the antibody, which is believed to bind to one side of the peptide-binding groove, directly affects the binding of peptide to the HLA-A2 molecule at the cell surface.

Experiments using anti-HLA class I monoclonal antibodies to inhibit target-cell lysis by virus-specific CTL have demonstrated the importance of the class I molecule in MHC-restricted recognition of antigen[4,5] Most allele-specific antibodies are thought to bind to the helices on either side of the putative peptide-binding groove of class I molecules[6], thereby possibly effecting the binding of peptide to the class I molecules

We investigated the effect of antibody MA2 1, a high-affinity antibody with specificity for HLA-A2 and HLA-B17 molecules[3,7] on the association of peptide with HLA-A2-expressing target cells, as detected by HLA-A2-restricted CTL According to the crystal structure of HLA-A2 (ref 6), the critical residues for recognition by MA2 1 antibody, residues 62-66 of the α1 domain[8], are in a region important for both T-cell recognition and peptide binding Residue 63, moreover, has an interdomain contact with residue 171 of the α_2-helix[9] We have previously reported that HLA-A2-restricted CTL specific for matrix protein of the influenza A virus recognize the synthetic peptide representing residues 58-68 of matrix protein (M58-68)[10] Some of these CTL are also able to recognize synthetic peptide in association with HLA-Aw69, encoded by an allele closely related to that of HLA-A2, but which does not bind MA2 1 antibody[11]

Surprisingly, when added directly to a cytotoxicity assay, the MA2 1 antibody greatly enhanced the recognition of peptide M58-68 on autologous target cells by peptide-specific HLA-A2/Aw69 cross-restricted CTL (Fig 1*a*) This effect was consistently seen at antibody concentrations greater than 0 1 $\mu g\ ml^{-1}$ On a target cell expressing HLA-Aw69 and HLA-B57, however, no enhancement of recognition by the same CTL was seen (Fig 1*b*), even though the antibody still bound to both T cells and target cells Another HLA-A2-specific monoclonal antibody, PA2 1 (ref 12, cross-reactive with HLA-Aw69) did not show any shift in the dose-response curve for the recognition of peptides on target cells expressing either HLA-A2 or HLA-Aw69 (Fig 1*a*, *b*) The slight inhibition of recognition shown in Fig 1*b* with MA2 1 antibody could be due to effects on the T cell, because incubation of HLA-A2-restricted CTL in high concentrations of MA2 1 antibody can inhibit their ability to lyse target cells (data not shown) The enhancement is not unique to this peptide, as similar effects have been shown for the recognition of a different peptide, from the nucleoprotein of influenza B virus, recognized by specific HLA-A2-restricted CTL (data not shown, and P Robbins, personal communication)

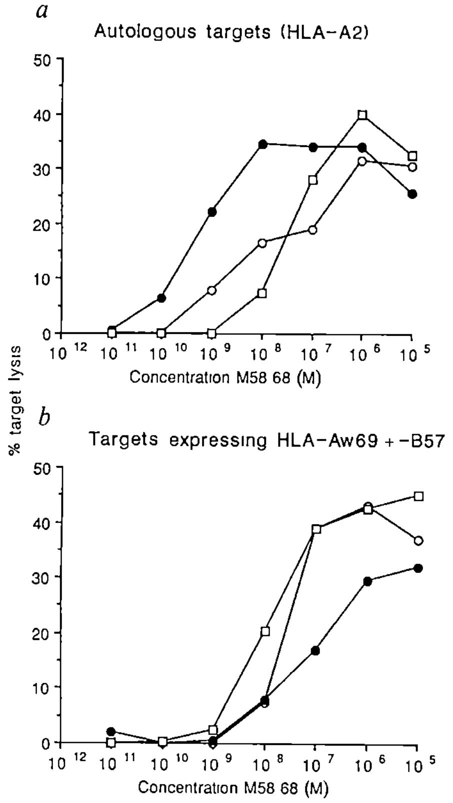

FIG 1 The anti-HLA-A2 antibody, MA2 1, enhances recognition of peptide by matrix-specific HLA-A2/Aw69 cross-restricted CTL on HLA-A2—but not HLA-Aw69—expressing target cells Effect of the presence of antibodies MA2 1 or PA2 1 on the dose-response curve of recognition of the peptide M58-68 of influenza matrix protein by CTL clone 1A10 from the donor JM (A2, 2, B15, 51 DR4, 4) on *a*, autologous target cells or *b*, a target cell MAL-NF (A1, w69, B35, 57, DR7, 10) which binds MA2 1 antibody on its B57 allele product Both peptide and antibody (1 25 $\mu g\ ml^{-1}$) were added directly to the assay Whereas MA2 1 antibody enhanced recognition of peptide by 10- to 100-fold on autologous cells, a small degree of inhibition was seen with the HLA-Aw69 expressing cells Antibody PA2 1 did not produce a shift in dose-response curve in either case Killer target ratio (K T)=1 8 Lysis in the absence of peptide, <2 3% ○, No antibody, ●, MA2 1, □, PA2 1

METHODS Influenza A virus matrix peptide M58-68 (Gly-Ile-Leu-Gly-Phe-Val-Phe-Thr-Leu-Thr-Val) was synthesized and donated by Dr J Rothbard (ICRF, London) CTL clone 1A10 was derived as previously described[11] Purified monoclonal antibodies MA2 1 (ref 3) and PA2 1 (ref 12) were used, and were donated by Dr J G Bodmer (ICRF, London) ^{51}Chromium-release cytotoxicity assay Epstein-Barr virus-transformed lymphoblastoid cells (BCL), used as target cells, were labelled with ^{51}Cr and added to the assay with CTL and diluted peptide and antibody, where appropriate, for 4 h in round-bottomed 96-well plates Percentage target lysis was calculated from the formula (E − M/D − M) × 100 E, experimental ^{51}Cr release, M, release in presence of culture medium (always <18%), D, release by 5% Triton X-100

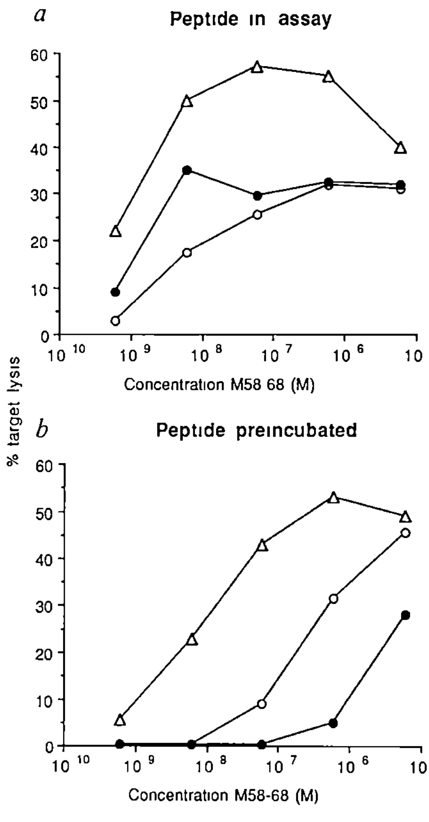

FIG 2 Optimum enhancement of recognition by MA2 1 antibody occurred when target cells were exposed to antibody before or during exposure to peptide, and when MA2 1 antibody was not present in the assay MA2 1 antibody inhibited recognition of target cells which had been pretreated with peptide CTL clone 1A10-recognition of peptide M58-68 on autologous BCL target cells (K T=3) was affected differently depending on the order of exposure of BCL to MA2 1 antibody or peptide *a*, Peptide M58-68 titrated in the assay at the concentrations shown, target cells were either untreated (○) or pretreated with MA2 1 antibody (1 $\mu g\ ml^{-1}$) (△), MA2 1 antibody added directly to the assays (●) Lysis in the absence of peptide, <1% *b*, Target cells were pretreated with peptide M58-68 at the concentrations shown ○, No antibody, △, simultaneous pretreatment with MA2 1 antibody and peptide, ●, BCL pretreated with peptide with MA2 1 antibody added directly to assay

METHODS As in Fig 1, except that target cells pretreated with peptide were incubated, in dilution of peptide shown, for 1 h and then washed three times before inclusion in the assay

These results show that the enhancement of peptide recognition by MA2 1 antibody is due to binding to the peptide-presenting HLA-A2 of the target cell rather than to HLA-A2 on the T cell, or to an irrelevant allele product (that is, non-presenting) on the target cell To optimize the enhancement, various conditions of exposure to peptide and antibody were investigated Figure 2*a* shows the results of experiments in which peptide was titrated in the CTL assay, and target cells were either pretreated with antibody, or antibody was added directly to the assay Pretreatment with antibody enhanced peptide recognition ~100-fold with greatly increased lysis, whereas adding antibody to the assay only gave a modest increase Figure 2*b* shows the results of experiments in which target cells were pretreated with peptide, and antibody was either added with the peptide or added directly to the assay Whereas pretreatment with antibody again produced nearly 100-fold enhancement of peptide recognition, addition of MA2 1 antibody to the assay inhibited the recognition of the peptide-treated target cells, similar to the inhibition of recognition of virus-infected cells (refs 4 and 5, and data not shown) Thus, MA2 1 antibody has a dual action, first, it enhances peptide association at the level of HLA-A2 on the target cell, and second, it interferes with T-cell recognition when it is present in the assay

Because both MA2 1 and PA2 1 are IgG1 monoclonal antibodies[13], and only MA2 1 antibody enhances peptide recognition, it is unlikely that the IgG1 Fc receptor mediates the effect As antibody divalency could be important, either by crosslinking target cells and CTL, or by crosslinking HLA-A2 on the target-cell surface, the effects of F(ab')$_2$ and Fab' fragments of MA2 1 antibody were assessed Target cells were pretreated with MA2 1 antibody or its fragments, using PA2 1 antibody as a control CTL (Fig 3*a*) were able to recognize peptide at 1,000-fold lower concentrations on HLA-A2-matched target cells pretreated with either whole MA2 1 antibody or its F(ab')$_2$ fragment relative to control The enhancement seen with Fab' was much less, although consistent in five different experiments, and could be due to lower avidity of the monovalent fragment[7] But a 10-fold enhancement of peptide recognition was seen with target cells incubated simultaneously with peptide and Fab' (Fig 2*b*) This effect is unlikely to be due to contamination of the sample with divalent fragments, because SDS-PAGE of a radio-iodinated sample did not show any trace of either whole IgG or F(ab')$_2$ (Fig 3*c*)

The simplest explanation of the data presented here is that the presence of MA2 1 antibody on a target cell enables HLA-A2 to bind peptide at the cell surface more efficiently To test this hypothesis, the kinetics of target-cell sensitization with peptide were studied on untreated and MA2 1 antibody-pretreated cells Targets were pulsed with 5 µM peptide (Fig 4*a*) in the absence of antibody, maximum lysis was not reached by 20 min of incubation with peptide But when the target cell was saturated in antibody, a 30-s peptide pulse was sufficient to obtain almost maximal lysis The lower concentration of 0 05 µM peptide was insufficient to give any lysis in the absence of antibody, but with the antibody-treated cells, the lysis was as effective as it was at the 100-fold greater peptide concentration in the absence of antibody

The rapidity of target sensitization in the presence of antibody

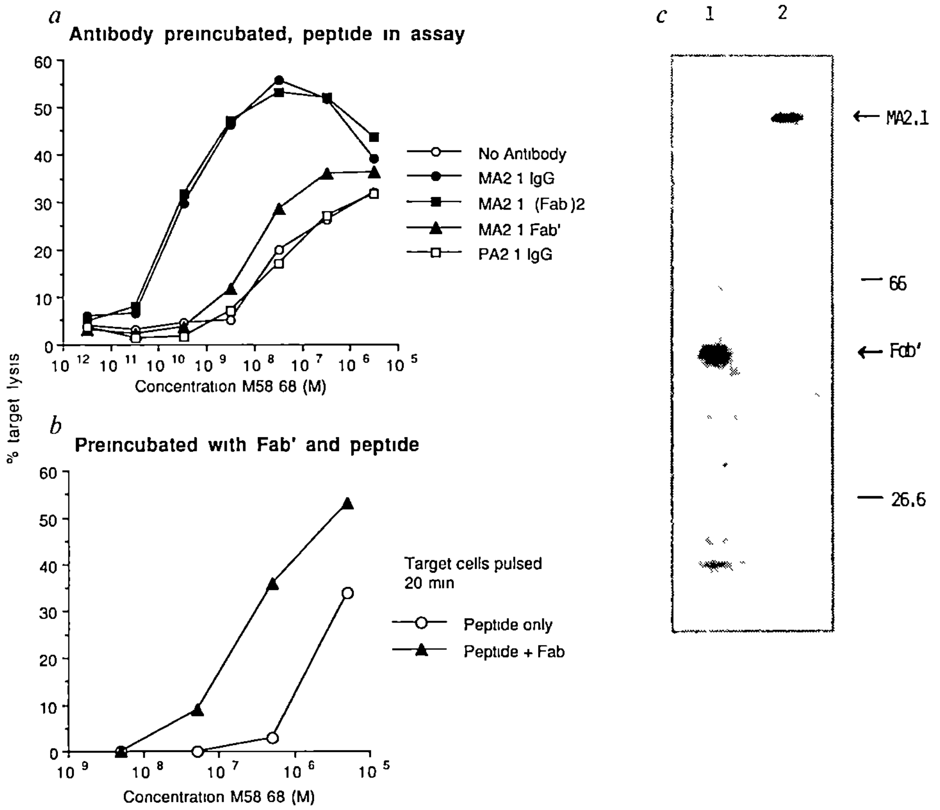

FIG 3 Fragments of MA2 1 antibody are able to enhance recognition of peptide Effect of MA2 1 antibody or its Fab' or F(ab')$_2$ fragments (1 µg ml^{-1}, that is, equivalent numbers of combining sites for Fab' and F(ab')$_2$) on the dose–response curve of recognition of peptide M58–68 of the polyclonal CTL line JM 22 on HLA-A2-matched BCL target cells (A2, 11, B54, 0, DR4, 12) K T=2 Lysis in the absence of peptide, <3 5% *a*, BCL target cells were pretreated in antibody MA2 1 or PA2 1 or fragment (1 µg ml^{-1}) as indicated and washed before inclusion in the assay Both MA2 1 antibody and its F(ab')$_2$ fragment caused a 100- to 1,000-fold enhancement of recognition, the Fab' fragment enhanced recognition by ~10-fold No enhancement was seen after preincubation with PA2 1 *b*, Recognition of autologous target cells by JM 22CTL was enhanced 10-fold (K T=2) after pretreatment of the target cells with a simultaneous pulse of peptide and Fab' (5 µg ml^{-1}) Target cells were either pulsed with peptide alone at the concentrations shown, or with peptide with MA2 1 Fab', for 20 min, followed by three washes before addition to the assay No free peptide or antibody fragment was added to the assay *c*, SDS-PAGE analysis of iodinated samples of the Fab' fragment of MA2 1 antibody (lane 1) or whole MA2 1 antibody (lane 2), 10% polyacrylamide gel, under nonreducing conditions Relative molecular mass markers indicated (×10^{-3})

METHODS JM 22 CTL were derived and maintained as previously described for other polyclonal matrix-specific lines[10] F(ab')$_2$ and Fab' fragments of MA2 1 antibody were prepared from purified antibody by pepsin digestion, according to the method previously described for mouse IgG1 monoclonal antibodies[13] Purity of fragments was established by SDS-PAGE (see *c*, and data not shown) Fragments were still able to bind HLA-A2 specifically as indicated by indirect fluorescence binding (data not shown) ^{51}Cr release assay As for Fig 1 except for *a*, BCL target cells were pretreated in antibody or fragments for 1 h and then washed three times before inclusion in the assay *b*, Pretreatment of cells was for 20 min SDS-PAGE of iodinated Fab' and whole antibody Whole antibody or Fab' fragment (~10 µg) was iodinated by addition of ^{125}I (0 5 µCi) in Chloramine-T (2 4 g ml^{-1}) for 2 min The reaction was stopped with saturated tyrosine, and the iodinated fraction was separated on a PD-10 column Each sample (10^6 c p m) were analysed on a 10% acrylamide gell by SDS-PAGE under nonreducing conditions The gel was fixed, stained, dried and exposed to X-ray film makes an interaction at the cell surface a likely explanation for the enhancement of peptide recognition This is supported by the fact that pretreatment of target cells with MA2 1 antibody before virus infection does not enhance the recognition of these cells (data not shown) Up-regulation of expression of class I molecules or HLA-A2 is an unlikely explanation, both because cycloheximide (an inhibitor of protein synthesis) has no effect on the enhancement, and antibody-binding studies have not shown an increase in cell surface HLA-A2 after MA2 1 antibody treatment (data not shown) The experiment represented in Fig 1*b* rules out the crosslinking of T cell and target as a possible explanation

The cell-surface effect could result from an increase in the number of peptide molecules bound in an immunogenic conformation, or from an increase in the total number of peptide molecules bound to HLA-A2 If, as predicted by the critical binding residues 62–66, MA2 1 antibody binds at the side of the groove in HLA-A2, the antibody could stabilize a conformation of A2 that allows freer exchange of peptide As the T-cell receptor probably binds at a similar site, it is possible that the receptor could also facilitate antigenic peptide exchange This could be an important factor in the dissociation of the T-ce receptor–peptide–MHC complex once the T cell has bee triggered

These experiments demonstrate that peptide can rapid associate with HLA-A2 at the surface of the cell in the presen of MA2 1 The antibody MA2 1 could be a useful tool in th preparation of a specific peptide with HLA-A2 for structur studies

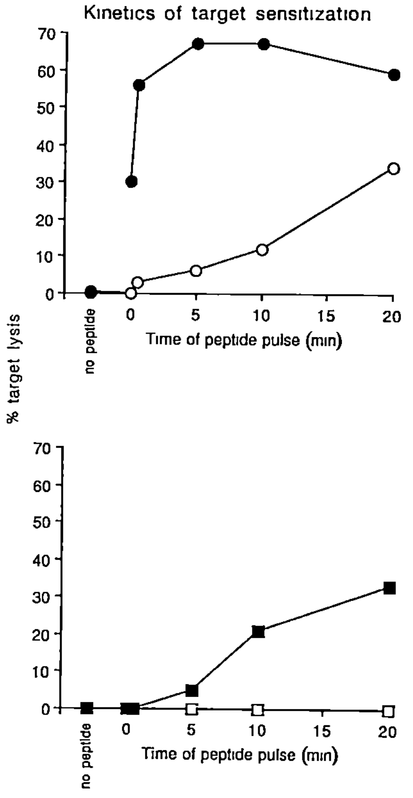

FIG 4 Target sensitization with peptide occurs much more rapidly and at lower concentrations of peptide on target cells for target cells pretreated with MA2 1 antibody (●, ■) compared with untreated cells (○, □) Lysis by JM 22 CTL (K T=2) of autologous JM BCL target cells which either had been pretreated with MA2 1 (5 μg ml^{-1}) or had not been treated, were pulsed with peptide M58-68 (5 μM above, 0 05 μM, below) for various time intervals from 30 s to 20 min

METHODS JM 22 CTL and ^{51}Cr-release assay as described in other figure legends, except for preparation of target cells, which were labelled with ^{51}Cr and either treated with MA2 1 (5 μg ml^{-1}) or not treated, for 1 h Washed cells were then pulsed with peptide for the appropriate time interval followed by an immediate 100-fold dilution in serum-free medium at 4 °C and then washed twice in RPMI-1640 at 4 °C followed by a further wash in medium containing 10% FCS at room temperature Cells were then diluted and added to the assay in the usual way Time zero represents the lysis due to peptide which associated with the cells as a result of the 100-fold dilution of peptide M58–68 in the first wash at 4 °C Spontaneous release of ^{51}Cr in the absence of CTL was <17%

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1 Townsend A R M *et al* *Cell* **44**, 959–968 (1986)
2 Townsend A R. M. *et al* *Nature* **340**, 443–448 (1989)
3 McMichael A J Parham P Rust, N & Brodsky F M *Hum Immun* **1**, 121–129 (1980)
4 McMichael A J Parham P Brodsky F M & Pilch J R *J exp Med* **152**, 195s–203s (1980)
5 Dongworth D W & McMichael A J *Br med Bull* **40**, 254–261 (1984)
6 Bjorkman P J *et al* *Nature* **329**, 512–518 (1987)
7 Ways J P & Parham P *Biochem J* **216**, 423–432 (1983)
8 Santos Aguado J Barbosa J A Biro P A & Strominger J L *J Immun* **141**, 2811–2818 (198
9 Bjorkman P J *et al* *Nature* **329**, 506–512 (1987)
10 Gotch F M *et al* *Nature* **326**, 881–882 (1987)
11 Bodmer H. C Gotch F M & McMichael A J *Nature* **337**, 652–655 (1989)
12 Parham P & Bodmer W F *Nature* **276**, 397–399 (1978)
13 Parham P *J Immun* **131**, 2895–2902 (1983)

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Insertion of cutinase gene into a wound pathogen enables it to infect intact host

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MANY phytopathogenic fungi must breach the intact plant cuticle to successfully invade and colonize their hosts. It has been suggested that cutinase, an extracellular enzyme secreted by many fungi, is essential for infection in some host–pathogen interactions Chemical or immunological inhibition of cutinase protects the host from infection[1,2]. We have cloned and characterized the cutinase complementary DNA and gene from *Fusarium solani* f. sp. *pisi*[3,4], a pea pathogen. A construct containing the cutinase coding region and extensive portions of the 5′ and 3′-flanking regions from the *Fusarium* genome was transferred into another phytopathogenic Ascomycete, *Mycosphaerella* spp , a parasitic fungus that affects papaya fruits only if the fruit skin is mechanically breached before inoculation[5]. Here we describe the introduction of the *Fusarium* cutinase gene into *Mycosphaerella* to yield transformants in which this gene is inducible by cutin hydrolysate. We demonstrate that these transformants of the wound-requiring fungus have the capacity to infect intact papaya fruits, and that this infection can be prevented by antibodies against *Fusarium* cutinase.

Cutin, a biopolyester, is the chief structural component of plant cuticle[6] Fungal contact with this polymer acts as a signal to induce cutinase production by the spores[7] Chemically prepared cutin hydrolysate can also induce the cutinase gene, transcripts being detectable within fifteen minutes after the addition of inducer[7] This induction allows the fungus to breach the cuticular barrier and penetrate into the host *Mycosphaerella* spp is unable to penetrate the outer defensive barrier of papaya fruits but can infect the fruits through wounds To test whether this limitation of the pathogen could be overcome by the acquisi-

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Our chief finding, therefore, is that an absence of CETP results rom a single base change in the conserved splice donor site in subject (S.Y.) and his sister (K.H.), both of whom have narkedly increased and enlarged HDL. Furthermore, there were educed levels of CETP in the children, obligate heterozygotes vho carry the defect. They also have about half the normal evels of CETP and moderately increased or enlarged HDL. The nagnitude of the increase in HDL (4-6 times normal) in S.Y. nd his sister K.H. is surprising, and indicates that CETP is of najor importance in HDL catabolism, influencing the size, omposition and quantity of HDL. The mechanism leading to he increase in the HDL protein apoA-I in S.Y. and K.H. (Table) is unclear, but could result from slower catabolism of their greatly enlarged HDL particles. It is also notable that low-density lipoproteins (LDL) were decreased in S.Y. and K.H.[4], which is shown by a lack of Sudan Black staining in the LDL region (Fig. 2*a*). This finding indicates that the normal formation of LDL requires CETP. There are no obvious clinical manifestations of CETP deficiency in S.Y., his sister or the children[4]. The lipoprotein profile associated with human CETP deficiency (that is, high HDL and low LDL) has low atherogenic potential, raising the possibility that CETP inhibitors could be used as anti-atherogenic drugs. Indeed, the lipoprotein profile found in human CETP deficiency resembles that of species that lack CETP activity in their plasma (rats, dogs and pigs) and which are relatively resistant to atherosclerosis[2]. □

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1. Breslow, J. L. in *Metabolic Basis of Inherited Disease* 6th ed., 1251–1266 (McGraw-Hill, New York, 1989).
2. Tall, A. R. *J. Lipid Res.* **27,** 361–367 (1986).
3. Padgett, R. A., Grabowski, P. J., Konarska, M. M., Seilea, S. & Sharp, P. A. *A. Rev. Biochem.* **55,** 1119–1150 (1986).
4. Koizumi, J. *et al. Atherosclerosis* **58,** 175–186 (1985).
5. Morton, R. E. & Zilversmit, D. B. *J. biol. Chem.* **256,** 11992-11995 (1981).
6. Barter, P. J. & Jones, M. E. *J. Lipid Res.* **21,** 238–249 (1980).
7. Hesler, C. B., Swenson, T. L. & Tall, A. R. *J. biol. Chem.* **262,** 2275–2282 (1987).
8. Hesler, C. B. *et al. J. biol. Chem.* **263,** 5020–5023 (1988).
9. Swenson, T. L. *et al. J. biol. Chem.* **264,** 14318–14326 (1989).
0. Marcel, Y. L. *et al. Arteriosclerosis* **8,** 593*a* (1988).
1. Drayna, D. *et al. Nature* **327,** 632–634 (1987).
2. Saiki, R. *et al. Science* **239,** 487–491 (1988).
13. Hattori, M. & Sakaki, Y. *Analyt. Biochem.* **152,** 232–238 (1986).
14. Mount, S. M. *Nucleic Acids Res.* **10,** 459–472 (1982).
15. Green, M. R. *A. Rev. Genet.* **20,** 671–708 (1986).
16. Aebi, M., Hornig, H., Padgett, R. A., Reiser, J. & Weissman, C. *Cell* **47,** 555–565 (1986).
17. Lamond, A. I., Konarksa, M. M., Grabowski, P. J. & Sharp, P. A. *Proc. natn. Acad. Sci. U.S.A.* **85,** 411–415 (1988).
18. Treisman, R., Orkin, S. H. & Maniatis, T. *Nature* **302,** 591–596 (1983).
19. Baird, M. *et al. Proc. natn. Acad. Sci. U.S.A.* **78,** 4218–4221 (1981).
20. Wieringa, B., Meyer, F., Reiser, J. & Weissmann, C. *Nature* **301,** 38–43 (1983).
21. Mitchell, P. J., Urlaub, G. & Chasin, L. *Molec. Cell Biol.* **6,** 1926–1935 (1986).
22. Bird, A. P. *Nucleic Acids Res.* **8,** 1499–1504 (1980).
23. Coulondre, C., Miller, J. H., Farabaugh, P. J. & Gilbert, W. *Nature* **274,** 775–780 (1978).
24. Kunkel, L. M. *et al. Proc. natn. Acad. Sci. U.S.A.* **74,** 1245–1249 (1977).
25. Higuchi, R., von Beroldingen, C. H., Sensabauth, G. H. & Erlich, H. A. *Nature* **332,** 543–545 (1988).
26. Maxam, A. M. & Gilbert, W. *Meth. Enzym.* **65,** 449–560 (1980).

Demonstration by genetic suppression of interaction of GroE products with many proteins

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THE way in which proteins attain and maintain their final form s of fundamental importance. Recent work has focused on the role of a set of ubiquitous proteins, termed chaperonins[1], in the ssembly of phage[2] and multisubunit proteins[3,4]. The range of haperonin action is unknown; they could interact with most ellular polypeptides or have a limited subset of protein partners. Included in the chaperonin family is the essential heat-shock regulated *Escherichia coli groEL* gene product[1,5,6]. Overexpression of the *groE* operon in *E. coli* causes enhanced assembly f heterologously expressed ribulose bisphosphate carboxylase subunits[3] and suppresses the heat-sensitive mutant phenotype of several *dnaA* alleles[7,8]. It has been inferred that suppression of eat-sensitive mutations is confined to *dnaA* alleles and that this onfinement could reflect an interaction between the *groE* operon roducts and a *dnaA* protein aggregate at the replication origin[7,9]. We now report that multiple copies of the *groE* operon suppress mutations in genes encoding several diverse proteins. Our data indicate a general role for the *groE* operon products, the GroEL and GroES proteins[10], in the folding–assembly pathways of many proteins.

It has been proposed that complexes of enzymes involved in sequential metabolic reactions are common in cells[11]. To investigate if enzymes of a biosynthetic pathway are part of a weakly associated *in vivo* supramolecular structure, we isolated heat-sensitive mutations in the major *ilv* operon of *Salmonella typhimurium* (D. Smulski, T.K.V.D. and R.A.L., unpublished results) that confer a conditional growth requirement for isoleucine and/or valine. This was achieved by using a localized hydroxylamine mutagenesis protocol[12]. It was expected that overexpression of the *groE* operon products might be able to correct growth defects by forcing either the folding or assembly of recalcitrant mutant polypeptides. The auxotrophic requirements of one mutation in *ilvGM* and two mutations in *ilvE*, encoding the multimeric enzymes acetolactate synthase II and transaminase B, respectively, were suppressed by the multicopy plasmid bearing the *groE* operon (Table 1). These data indicated that mutations in genes encoding biosynthetic enzymes can be suppressed by overexpression of the *groE* operon products.

We then investigated whether overexpression of the *groE* operon products could suppress mutations in the histidine (*his*) operon of *S. typhimurium* (Table 1). Heat-sensitive[13,14], cold-sensitive[14] and temperature-independent[13] auxotrophic missense mutations in the *his* operon were examined. Many, but not all, heat-sensitive *hisD*, *hisC* and *hisB* alleles were suppressed by *groE* over-expression (Table 1); these genes encode, respectively, histidinol dehydrogenase, histidinol-phosphate aminotransferase and the bifunctional protein imidazoleglycerol phosphate dehydratase—histidinol phosphate phosphatase[15]. Suppression of cold-sensitive and temperature-independent alleles has so far not been observed in the *his* system (Table 1).

We quantitated the suppression of the *hisD6592* allele by *groE* overexpression with a sensitive radiochemical assay[16], performed at 37 °C, of histidinol dehydrogenase activity present in toluene-permeablized cells grown at the restrictive temperature of 37 °C in minimal medium E (ref. 17) supplemented with excess (0.64 mM) L-histidine. Equivalent repressed levels of histidine biosynthetic enzymes are produced in both auxotrophs and prototrophs under these conditions[15]. Specific activities thus reflect differences unrelated to transcription. A strain containing the *hisD6592* allele and the control plasmid pTG10, had undetectable enzyme activity (<0.5% of wild-type specific activity) whereas a strain containing the same *hisD* allele and the plasmid overexpressing *groESL* (ref. 3) contained 3%

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TABLE 1 Suppression of missense alleles

Alleles	Type	Restrictive temperature (°C)	Suppression
S. typhimurium			
hisF8611[*] *hisF8612, hisF8613, hisH8606*	CS	18	−
hisB8435	HS	42	+
hisC6595, hisD141, hisD6585, hisD6586, hisD6589, hisD6591, hisD6592, hisD6593, hisC15	HS	37	+
hisC163	HS	30	+
hisC6596, hisC6598, hisC6599	HS	42	−
hisA8437, hisA8438, hisB8434, hisC6594	HS	39	−
hisB59, hisB59	HS	37	−
hisG6582, hisG6583	HS	30	−
hisA338, hisB14, hisB20, hisB53, hisC201, hisC899, hisD362, hisG46, hisG204, his I352	TI	NA	−
ilvE3149[†], *ilvE3150*	HS	37	+
ilvGM3139	HS	30	+
ilvGM3136, ilvGM3142, ilvGM3143, ilvGM3147, ilvGM3148	HS	42	−
ilvGM3137	HS	37	−
ilvGM3135, ilvGM3138, ilvGM3140, ilvGM3141	HS	30	−
S. typhimurium phage P22			
9tsfU3[‡], *9tsfU8*[§], *9tsfU34*[‡], *9tsfH304*[§],	HS	39	+
9tsfU38[§], *9tsfN39*[§], *9tsfU40*[§]	HS	39	−
9tsfUT51 9su334[§], *9tsfH302*[§]	HS	37	−
9tsfUT51[§]	HS	30	
E. coli			
secA51[‖], *secY24*[¶],	HS	42	+
alaS4[#], *glnSl*[#], *leuS31*[#], *metG83*[#], *serS14*[#]	HS	42	−

Plasmids pTG10 (control)[3] and pGroESL (*groES*$^+$ *groEL*$^+$)[3] were transformed into a restriction-deficient strain of *S. typhimurium* LT2, TR5878 (ref. 28) selecting for chloramphenicol resistance. Phage P22-mediated generalized transduction[17,29] was used to transfer the plasmid to the various *S. typhimurium* mutants. Growth of the *S. typhimurium* recombinants was tested at 18, 30, 37, 39 and 42 °C on minimal medium E supplemented with 0.4% glucose[17]. Introduction of pTG10 did not allow growth of any mutant at the restrictive temperature. (+), Strain containing plasmid pGroESL grew at temperatures restrictive for the growth of the otherwise isogenic plasmid pTG10 containing strain. Suppression of P22 mutations was tested by assaying plaque formation on lawns of *S. typhimurium* DB7136 (ref. 30) derivatives containing either plasmids pTG10 or pGroESL incubated at the restrictive temperature. Transformation was used to introduce the plasmids into *E. coli* strains. With the exception of *secY24*, suppression tests of all *E. coli* mutations was scored on chloramphenicol-supplemented LB plates[17] at 42 °C. For *secY24*-bearing *E. coli* strains[21], suppression tests were performed with the *groES*$^+$*EL*$^+$-bearing plasmid pOF39 (ref. 7) on minimal M9 medium[17] supplemented with glucose and ampicillin. Each *S. typhimurium* and *E. coli* strain contained a wild-type chromosomal copy of the *groE* operon. Abbreviations: CS, Cold-sensitive; HS, heat-sensitive; TI, temperature-independent; NA, not applicable.

[*] The *his* alleles, numbered below 1,000 are reviewed in ref. 13 whereas those numbered above 1,000 are described in ref. 14.

[†] The *ilv* alleles have not been reported previously.

[‡] Ref. 23.

[§] Ref. 22.

[‖] Ref. 20.

[¶] Ref. 21.

[#] Ref. 18.

of the activity present in the wild-type strain containing the plasmid overexpressing *groESL*.

All but one of the enzymes involved in histidine biosynthesis are multimeric[15]. Only two heat-sensitive alleles of *hisA* (ref. 14), specifying the monomeric protein phosphoribosylformimino-5-amino-1-phosphoribosyl-4-imidazolecarboxamide isomerase[15] exist; neither was suppressed by the multicopy *groE*-bearing plasmid (Table 1). Heat-sensitive mutants caused by alteration of monomeric proteins, although rare, have been described for several aminoacyl-tRNA synthetases in *E. coli*[18]. The proteins of this family display diverse quaternary structures (α, α_2, $\alpha\beta$, $\alpha_2\beta_2$, α_4)[19]. The *groE*-bearing plasmid did not suppress heat-sensitive growth defects caused by mutations in *alaS*, *glnS*, *leuS*, *metG* and *serS*, which are structural gene encoding, respectively, alanyl-(α_4), glutaminyl-(α), leucyl-(α) methionyl-(α_2) and seryl-(α_2) tRNA synthetases in *E. co* (Table 1).

Protein translocation involves interactions among sever components. Among mutations affecting the mitochondrial protein import machinery in *Saccharomyces cerevisiae* are allele of *MIF4*, which encodes a protein homologue of *E. coli* GroEL[4] We tested the ability of the *groE*-bearing plasmid to suppres heat-sensitive growth (Fig. 1; Table 1) and secretion (A.A.G. unpublished data) defects caused by the *secA51*[20] and *secY24*[2] alleles of *E. coli*. Suppression of both alleles was observed (Table 1), indicating that the suppression mechanism is gene-independent in *E. coli* as well as in *S. typhimurium*. The presence o pGroESL had no effect on the growth of a strain containing conditionally lethal amber mutation in gene *X* upstream of *sec* (A.A.G., unpublished data); the lethality is due to the pola effect of the mutation on *secA*, resulting in the absence of th *secA* protein[20]. This implies that the *secA* protein must be presen for the suppression of the secretion defect and that *groE* overexpression cannot bypass this requirement.

Can mutations altering structural proteins, rather tha enzymes, be suppressed by overexpression of the *groE* operon products. Gene *9* of *Salmonella* phage P22 encodes a polypeptide that trimerizes to form the phage's tail spike, six of which are attached to each phage particle[22]. Many extensively characterized heat-sensitive protein-folding mutations of gene *9* exist[22,23]. Phages containing such mutations do not plaque on wild-type *S. typhimurium* at the restrictive temperature. We determined the ability of these mutants to form plaques on strains containing either a control or the *groE* multicopy plasmid at the restrictive temperature (Table 1). Several mutations were suppressed.

We characterized the suppression of the P22 *9tsfH304* mutation more fully. The efficiency of plating of this mutant at 39 °C versus 30 °C on a strain containing a control plasmid[3] was less than 10^{-4}. By contrast, the efficiency of plating on a host containing the plasmid overexpressing *groESL* (ref. 3) was 0.1. At 30 °C we observed a burst size of ~200 with either host. Infection of the strain overexpressing *groESL* with the mutant phage resulted in a burst of six plaque-forming units (PFU) at 39 °C; a burst of 0.4 PFU was observed when the host contained the control plasmid.

The nucleotide and corresponding deduced amino-acid sequences of the *groE*-suppressed phage P22 gene *9* (refs 22,

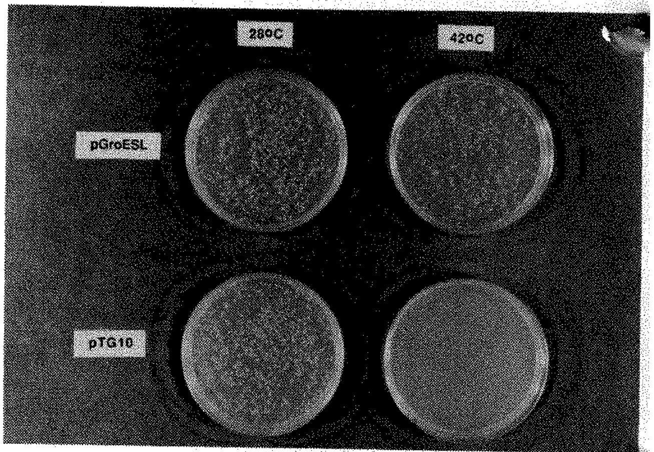

FIG. 1 Suppression of the heat-sensitive phenotype of a *secA51* strain. Strain MM52 (*secA51*)[20] was transformed with either plasmid pTG10 (*groES*$^-$*groEL*$^-$) or plasmid pGroESL (*groES*$^+$*groEL*$^+$) selecting for chloramphenicol resistance. Recombinants were plated for single colonies on antibiotic-containing LB medium at the indicated temperatures. The presence of plasmid pGroESL suppressed the heat-sensitive phenotype of the *secA51* mutant.

TABLE 2 Nucleotide sequences and deduced amino-acid sequences of suppressible alleles

Allele	Codon change	Amino-acid change	Amino-acid residue	Ref.
secA51	CTG → CCG	Leu → Pro	43	20
secY24	GGT → GAT	Gly → Asp	240	21
P22 *9 tsfU34*	AAG → GAG	Lys → Glu	163	23
P22 *9 tsfH304*	GGA → AGA	Gly → Arg	244	22
P22 *9 tsfU3*	GAA → GTA	Glu → Val	309	23
P22 *9 tsfU8*	GAA → AAA	Glu → Lys	344	23

The *E. coli secA*, *secY* and the phage P22 gene *9* proteins are of 901, 443 and 666 amino-acid residues, respectively[20-23].

23) and the *sec* alleles[20,21] are known (Table 2). At the protein level, these gene *9* transition and transversion mutations cause replacement of an acidic side chain by a basic one (glutamic acid → lysine) and a basic R-group by an acidic one (lysine → glutamic acid), loss of a negative charge (glutamic acid → valine) and substitution of a bulky positively charged side chain for a proton (glycine → arginine). The *secA* mutation substitutes the imino acid proline in place of leucine, whereas the *secY* allele results in the substitution of the acidic side chain of aspartic acid for the proton of glycine. Proposed suppression mechanisms must be consistent with the wide variety of suppressible alleles.

Suppression of *dnaA* alleles[7,8], phage λ morphogenesis[10], and ribulose bisphosphate carboxylase assembly[3], require both *groEL* and *groES*. Both *groE* genes were also needed to mediate suppression of the mutations used in our study. Plasmids expressing either *groEL* or *groES* were unable to suppress representative alleles of gene *9*, *hisD*, *hisC*, *ilvGM* and *secA* responsive to the intact *groESL* operon (Table 3).

There are several mechanisms by which multiple copies of *groE* could act as a general suppressor of heat-sensitive and perhaps other missense mutations. *GroE* operon products could alter the translational fidelity by their interaction with the ribosome[24], or reduce protein degradation by having an antiproteolytic role, or modulate transcription rate, translation rate or ionic strength[14]. But *groE*-mediated translational misreading is unlikely to be a common means of suppression, because misreading, induced by the presence of aminoglycoside antibiotics, does not allow growth of the *groE*-suppressible *hisC15* mutant[25]. Also, the broad spectrum of suppressible alleles (Table 2) precludes codon-specific mechanisms of informational suppression. And because protein degradation is decreased in a *groEL* mutant of *E. coli*[26], it is unlikely that GroE over-production antagonizes degradation. Elevated transcription or translation of target genes also cannot explain the observed suppression, because expression of the *Salmonella his* and *ilv* operons is not increased by the presence of the multicopy *groE*-bearing plasmid pGroESL (R.A.L., unpublished observations). Finally, the *groE*-suppressible *ilvGM3139* allele is not responsive to phenotypic suppression by sodium chloride supplementation[14] (T.K.V.D., unpublished observations), indicating that elevation of the internal ionic strength cannot be a common means of suppressing the *groE*-responsive heat-sensitive mutations. Thus, the explanation most consistent with present views[1,27] and our data is that overexpressed *groE* gene products directly interact with the mutant proteins, allowing them to achieve their active conformations. Such interactions could reflect a normal association of the GroE proteins with the translation products of many genes, including wild-type versions of the suppressed alleles. □

TABLE 3 Suppression requires *groES* and *groEL*

	Growth in the presence of:			
Allele	pTG10 (groES$^-$L$^-$)	pGroEL (groES$^-$L$^+$)	pGroES (groES$^+$L$^-$)	pGroESL (groES$^+$L$^+$)
hisC6595	−	−	−	+
hisD6592	−	−	−	+
ilvGM3139	−	−	−	+
secA51	−	−	−	+
P22 *9 tsfH304*	−	−	−	+

Plasmid pGroES was constructed by ligating the small *Eco*RI restriction fragment from plasmid pS4 (ref. 7) into the *Eco*RI site of the polylinker present in pTG10 (ref. 3). Plasmid pGroES and the other listed plasmids[3] were introduced into a variety of strains carrying the indicated chromosomal mutation and the host for phage P22 growth, DB7136, as described in the legend to Table 1. Conditions to test suppression were as described in Table 1.

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1. Hemmingsen S. M. *et al. Nature* **333,** 330–334 (1988).
2. Friedman D. I. *et al. Microbiol. Rev.* **48,** 299–325 (1984).
3. Goloubinoff P., Gatenby, A. A. & Lorimer, G. H. *Nature* **337,** 44–47 (1989).
4. Cheng, M. Y. *et al. Nature* **337,** 620–625 (1989).
5. Fayet, O., Ziegelhoffer, T. & Georgopoulos, C. *J. Bact.* **171,** 1379–1385 (1989).
6. Neidhardt, F. C. & van Bogelen, R. A. in Escherichia coli *and* Salmonella typhimurium (eds Neidhardt, F. C. *et al.*) 1334–1345 (American Society for Microbiology, Washington, D.C., 1987).
7. Fayet, O., Louarn, J.-M. & Georgopoulos, C. *Molec. Gen. Genet.* **202,** 435–445 (1986).
8. Jenkins, A. J., March, J. B., Oliver, I. R. & Masters, M. *Molec Gen. Genet.* **202,** 446–454 (1986).
9. McMacken, R., Silver, L. & Georgopoulos, C. in Escherichia coli *and* Salmonella typhimurium (eds Neidhardt, F. C. *et al.*) 564–612 (American Society for Microbiology, Washington, D.C., 1987).
10. Tilly, K., Murialdo, H. & Georgopoulos, C. *Proc. natn. Acad. Sci. U.S.A.* **78,** 1629–1633 (1981).
11. Srivastava, D. K. & Bernhard, S. A. *Science* **234,** 1081–1086 (1986).
12. Hong, J.-S. & Ames, B. N. *Proc. natn. Acad. Sci. U.S.A.* **68,** 3158–3162 (1971).
13. Hartman, P. E., Hartman, Z., Stahl, R. C. & Ames, B. N. *Adv. Genet.* **16,** 1–34 (1971).
14. Kohno, T. & Roth, J. *Biochemistry* **18,** 1386–1392 (1979).
15. Winkler, M. E. in Escherichia coli *and* Salmonella typhimurium (eds Neidhardt, F. C. *et al.*) 395–411 (American Society for Microbiology, Washington, D.C., 1987).
16. Ciesla, Z., Salvatore, F., Broach, J. R., Artz, S. W. & Ames, B. N. *Analyt. Biochem* **63,** 44–55 (1975).
17. Davis, R. W., Botstein, D. & Roth J. *Advanced Bacterial Genetics* (Cold Spring Harbor Laboratory, New York 1980).
18. LaRossa, R. & Soll, D. in *Transfer RNA* (ed. Altman, S.) 136–167 (MIT, Cambridge, Massachusetts, 1978).
19. Schimmel, P. *A. Rev. Biochem.* **56,** 125–158 (1987).
20. Schmidt, M. G., Rollo, E. E., Grodberg, J. & Oliver, D. B. *J. Bact.* **170,** 3404–3414 (1988).
21. Shiba, K., Ito, K., Yura, T. & Cerretti, D. P. *EMBO J.* **3,** 631–635 (1984).
22. Yu, M.-H. and King, J. *Proc. natn. Acad. Sci. U.S.A.* **81,** 6584–6588 (1984).
23. Villafane, R. & King, J. *J. molec. Biol.* **204,** 607–619 (1988).
24. Neidhardt, F. C. *et al.* A. R. *J. Bact.* **145,** 513–520 (1981).
25. Whitfield, Jr., H. J., Martin, R. G. & Ames, B. N. *J. molec. Biol.* **21,** 335–355 (1966).
26. Straus, D. B., Walter, W. A. & Gross, C. A. *Genes Dev.* **2,** 1851–1858 (1988).
27. Bochkareva, E. S., Lissin, N. M. & Girshovich, A. S. *Nature* **336,** 254–257 (1988).
28. Enomoto, M. & Stocker, B. A. D. *Virology* **60,** 503–514 (1974).
29. Sanderson, K. E. & Roth, J. R. *Microbiol. Rev.* **47,** 410–453 (1983).
30. Fane, B. & King, J. *Genetics* **117,** 167–171 (1987).

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Identification of a novel translation factor necessary for the incorporation of selenocysteine into protein

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DURING the biosynthesis of selenoproteins in both prokaryotes and eukaryotes, selenocysteine is cotranslationally incorporated into the nascent polypeptide chain[1,2] through a process directed by a UGA codon that normally functions as a stop codon[3-5]. Recently, four genes have been identified whose products are required for selenocysteine incorporation in *Escherichia coli*[6]. One of these genes, *selC*, codes for a novel transfer RNA species (tRNA^{UCA}) that accepts serine and cotranslationally inserts selenocysteine by recognizing the specific UGA codon[7]. The serine residue attached to this tRNA is converted to selenocysteine in a reaction dependent on functional *selA* and *selD* gene products[8]. By contrast, the *selB* gene product (SELB) is not required until after selenocysteyl-tRNA biosynthesis[8]. Here we present evidence indicating that SELB is a novel translation factor. The deduced

amino-acid sequence of SELB exhibits extensive homology with the sequences of the translation initiation factor-2 (IF-2) and elongation factor Tu (EF-Tu). Furthermore, purified SELB protein binds guanine nucleotides in a 1:1 molar ratio and specifically complexes selenocysteyl-$\mathrm{tRNA}^{\mathrm{UCA}}$, but does not interact with seryl-$\mathrm{tRNA}^{\mathrm{UCA}}$. Thus, SELB could be an amino acid-specific elongation factor, replacing EF-Tu in a special translational step.

Functional SELB protein is absolutely required for translation of the selenocysteine-specific UGA codon in the *fdhF* messenger RNA (ref. 6, and unpublished results). We first determined the nucleotide sequence of the *selB* gene (Fig. 1), and found that it potentially encodes a protein of relative molecular mass 68,800 (M_r 68.8K) consisting of 614 amino acids. Computer-assisted analysis of the amino-acid sequence revealed a striking similarity of the N-terminal 244 amino acids to the sequence of the two translation factors EF-Tu and IF-2 (Fig. 2). It is of interest that the homologous region between SELB and EF-Tu corresponds to the segment of primary structure that also is conserved between EF-Tu and IF-2. In contrast to all G proteins identified so far, SELB shows one unique deviation in its guanine-nucleotide-binding motif[9]—the universal asparagine residue (position 135 in EF-Tu), which forms a hydrogen bond with the keto group of the guanine ring[10], is replaced by threonine. Sequence similarity was also found between SELB and several other G proteins, particularly EF-G, LEPA and p21. In these cases, however, similarity is restricted to the first 120 amino acids in SELB, corresponding to the G domain of EF-Tu (data not shown).

EF-Tu and IF-2 share the property of binding guanine nucleotides and aminoacyl-tRNA. If it is assumed that this function is associated with the conserved domain, then it might be anticipated that SELB exhibits similar properties. To examine this possibility, the *selB* gene product was overproduced in a T7 promoter expression system and purified (K.F., K. P. Rücknagel and A.B., manuscript in preparation). We then used equilibrium dialysis for nucleotide-binding studies with purified SELB (Fig. 3). The SELB protein bound guanine nucleotides in a 1:1 molar ratio. The dissociation constants were calculated to be 10 μM for SELB-GDP and 1.7 μM for SELB-GTP (Fig. 3*a*, *b*). Binding is specific for guanine nucleotides, because the addition of a 10-fold excess of various other nucleotides did not result in competition for binding of $[^3\mathrm{H}]$GTP (Fig. 3*c*); binding of $[^3\mathrm{H}]$GDP could only be quenched by GTP (data not shown). Thus, SELB binds GDP with an affinity similar to that exhibited by IF-2 (ref. 11). This deviates significantly from the affinity of EF-Tu for GDP, which is three orders of magnitude greater[12]. The trend is reversed for GTP binding; SELB and EF-Tu have similar affinities for GTP, whereas the affinity of IF-2 for GTP is lower by a factor of ~100 (refs 11, 12).

To examine whether SELB also interacts with *selC*-encoded $\mathrm{tRNA}^{\mathrm{UCA}}$, we purified the latter from a strain carrying the gene on a multicopy plasmid[23]. We analysed complex formation by determining the protection of the tRNA by SELB either against alkaline hydrolysis of the aminoacyl-tRNA ester bond[13,14], or against enzymatic cleavage of the tRNA by RNase[15]. After ligation to $\mathrm{tRNA}^{\mathrm{UCA}}$, L-serine was converted *in vitro* to selenocysteyl-tRNA by using extracts from cells overproducing the *selA* and *selD* gene products[23]. We then attempted to chemically deacylate the recovered selenocysteyl-tRNA in the presence or absence of SELB. We found a significant degree of protection of the tRNA against hydrolysis in the presence of SELB. This effect was independent of the addition of guanine nucleotides (Fig. 4*a*). By contrast, we could not detect any protection from deacylation when seryl-$\mathrm{tRNA}^{\mathrm{UCA}}$ was used in the same assay (Fig. 4*b*). We also found no protection with any other aminoacyl-tRNA tested (data not shown). Similar results were obtained when we assayed complex formation using the RNase-protection method[15]. SELB specifically protected selenocysteyl-$\mathrm{tRNA}^{\mathrm{UCA}}$ (Fig. 4*c*, *d*). Again, this effect did not require the presence of guanine nucleotides.

```
 -50                    GATTTACGCT GCCTTGAAGA TGAGCAACGG TTTTTGGAGA TGTTGTTGA

   1  ATG ATT ATT GCG ACT GCC GGA CAC GTT GAC CAC GGC AAA ACA ACC TTA TTG CA
   1  MET Ile Ile Ala Thr Ala Gly His Val Asp His Gly Lys Thr Thr Leu Leu G

  55  GCG ATT ACT GGC GTA AAT GCT GAC CGT CTG CCG GAA GAA AAA AAG CGC GGC A
  19  Ala Ile Thr Gly Val Asn Ala Asp Arg Leu Pro Glu Glu Lys Lys Arg Gly ME

 109  ACC ATC GAT CTC GGC TAT GCC TAC TGG CCG CAG CCG GAT GGT CGC GTG CCT GG
  37  Thr Ile Asp Leu Gly Tyr Ala Tyr Trp Pro Gln Pro Asp Gly Arg Val Pro Gl

 163  TTT ATC GAC GTT CCC GGT CAT GAA AAG TTT CTT TCC AAC ATG CTG GCG GGC GT
  55  Phe Ile Asp Val Pro Gly His Glu Lys Phe Leu Ser Asn MET Leu Ala Gly Va

 217  GGT GGT ATC GAT CAC GCG CTG TTG GTG GTG GCG TGC GAT GAC GGC GTG ATG GC
  73  Gly Gly Ile Asp His Ala Leu Leu Val Val Ala Cys Asp Asp Gly Val MET Al

 271  CAG ACC CGT GAG CAT CTG GCG ATT TTG CAG CTG ACC GGT AAC CCG ATG CTG AC
  91  Gln Thr Arg Glu His Leu Ala Ile Leu Gln Leu Thr Gly Asn Pro MET Leu Th

 325  GTG GCG CTG ACC AAA GCC GAT CGC GTG GAC GAA GCG CGT GTT GAT GAG GTT GA
 109  Val Ala Leu Thr Lys Ala Asp Arg Val Asp Glu Ala Arg Val Asp Glu Val Gl

 379  CGC CAG GTA AAG GAG GTT CTG CGG GAA TAC GGT TTT GCT GAG GCA AAA CTG TT
 127  Arg Gln Val Lys Glu Val Leu Arg Glu Tyr Gly Phe Ala Glu Ala Lys Leu Ph

 433  ATC ACC GCA GCA ACC GAA GGT CGG GGA ATG GAT GCC CTG CGC GAG CAT CTG CT
 145  Ile Thr Ala Ala Thr Glu Gly Arg Gly MET Asp Ala Leu Arg Glu His Leu Le

 487  CAG TTG CCG GAA CGC GAG CAC GCC AGC CAA CAT AGT TTC CGC CTC GCG ATT GA
 163  Gln Leu Pro Glu Arg Glu His Ala Ser Gln His Ser Phe Arg Leu Ala Ile As

 541  CGC GCA TTT ACC GTA AAA GGT GCC GGG CTG GTC GTC ACC GGT ACG GCG TTA AGC
 181  Arg Ala Phe Thr Val Lys Gly Ala Gly Leu Val Val Thr Gly Thr Ala Leu Ser

 595  GGG GAA GTG AAG GTA GGC GAT TCA CTC TGG CTG ACT GGT GTA AAT AAA CCG ATG
 199  Gly Glu Val Lys Val Gly Asp Ser Leu Trp Leu Thr Gly Val Asn Lys Pro MET

 649  CGT GTA CGT GCG CTG CAT GCG CAA AAC CAG CCA ACA GAA ACC GCC AAT GCC GGC
 217  Arg Val Arg Ala Leu His Ala Gln Asn Gln Pro Thr Glu Thr Ala Asn Ala Gly

 703  CAG CGT ATC GCG CTT AAC ATC GCG GGT GAT GCG GAA AAA GAG CAG ATT AAC CGT
 235  Gln Arg Ile Ala Leu Asn Ile Ala Gly Asp Ala Glu Lys Glu Gln Ile Asn Arg

 757  GGC GAC TGG CTG CTT GCC GAT GTG CCG CCA GAG CCG TTC ACA CGG GTG ATT GTC
 253  Gly Asp Trp Leu Leu Ala Asp Val Pro Pro Glu Pro Phe Thr Arg Val Ile Val

 811  GAG CTT CAA ACC CAT ACA CCG CTG ACC CAG TGG CAG CCG CTG CAT ATT CAC CAC
 271  Glu Leu Gln Thr His Thr Pro Leu Thr Gln Trp Gln Pro Leu His Ile His His

 865  GCC GCC AGC CAC GTC ACG GGA CGC GTT TCA CTG CTG GAA GAT AAC CTT GCT G
 289  Ala Ala Ser His Val Thr Gly Arg Val Ser Leu Leu Glu Asp Asn Leu Ala Gl

 919  CTG GTC TTC GAC ACC CCG TTA TGG CTG GCA GAT AAC GAC CGC CTG GTA TTG CGC
 307  Leu Val Phe Asp Thr Pro Leu Trp Leu Ala Asp Asn Asp Arg Leu Val Leu Arg

 973  GAT ATC TCT GCC CGC AAC ACG CTG GCC GGA GCG CGC GTC GTG ATG CTT AAC CCG
 325  Asp Ile Ser Ala Arg Asn Thr Leu Ala Gly Ala Arg Val Val MET Leu Asn Pro

1027  CCG CGT CGC GGT AAA CGT AAG CCG GAA TAT CTG CAA TGG CTG GCG TCT CTT GCA
 343  Pro Arg Arg Gly Lys Arg Lys Pro Glu Tyr Leu Gln Trp Leu Ala Ser Leu Ala

1081  CGG GCG CAG AGC GAT GCC GAT GCG TTA TCT GTT CAT CTG GAA CGC GGC GCG GTT
 361  Arg Ala Gln Ser Asp Ala Asp Ala Leu Ser Val His Leu Glu Arg Gly Ala Val

1135  AAC CTT GCG GAT TTC GCC TGG GCG CGC CAG CTC AAC GGC GAA GGG ATG CGC GAA
 379  Asn Leu Ala Asp Phe Ala Trp Ala Arg Gln Leu Asn Gly Glu Gly MET Arg Glu

1189  TTG CTG CAA CAG CCT GGT TAT ATT CAG GCT GGT TAT AGC TTG TTG AAT GCG CCG
 397  Leu Leu Gln Gln Pro Gly Tyr Ile Gln Ala Gly Tyr Ser Leu Leu Asn Ala Pro

1243  GTT GCC GCC CGC TGG CAG CGG AAA ATT CTC GAC ACA TTA GCG ACT TAT CAT GAG
 415  Val Ala Ala Arg Trp Gln Arg Lys Ile Leu Asp Thr Leu Ala Thr Tyr His Glu

1297  CAA CAT CGC GAT GAA CCT GGC CCT GGG CGC GAA CGT CTG CGA CGT ATG GCG TTG
 433  Gln His Arg Asp Glu Pro Gly Pro Gly Arg Glu Arg Leu Arg Arg MET Ala Leu

1351  CCA ATG GAA GAT GAA GCG CTG GTA CTG TTG CTG ATT GAA AAG ATG CGC GAA AGC
 451  Pro MET Glu Asp Glu Ala Leu Val Leu Leu Leu Ile Glu Lys MET Arg Glu Ser

1405  GGC GAC ATC CAC AGC CAT CAC GGC TGG CTG CAT CTG CCA GAT CAC AAA GCG GGC
 469  Gly Asp Ile His Ser His His Gly Trp Leu His Leu Pro Asp His Lys Ala Gly

1459  TTC AGC GAA GAG CAG CAG GCC ATC TGG CAA AAA GCA GAG CCA CTG TTT GGT GA
 487  Phe Ser Glu Glu Gln Gln Ala Ile Trp Gln Lys Ala Glu Pro Leu Phe Gly Asp

1513  GAA CCG TGG TGG GTG CGT GAC CTG GCA AAA GAG ACG GGA ACC GAC GAG CAG GCA
 505  Glu Pro Trp Trp Val Arg Asp Leu Ala Lys Glu Thr Gly Thr Asp Glu Gln Ala

1567  ATG CGC CTG ACT CTA CGC CAG GCG GCG CAG CAA GGA ATA ATT ACC GCG ATC GTT
 523  MET Arg Leu Thr Leu Arg Gln Ala Ala Gln Gln Gly Ile Ile Thr Ala Ile Val

1621  AAA GAT CGT TAT TAC CGT AAC GAT CGG ATT GTC GAG TTT GCC AAT ATG ATC CGC
 541  Lys Asp Arg Tyr Tyr Arg Asn Asp Arg Ile Val Glu Phe Ala Asn MET Ile Arg

1675  GAT CTC GAT CAG GAG TGT GGT TCA ACC TGC GCG GCG GAT TTC CGC GAT CGC TTA
 559  Asp Leu Asp Gln Glu Cys Gly Ser Thr Cys Ala Ala Asp Phe Arg Asp Arg Leu

1729  GGC GTA GGC CGA AAG CTG GCA ATT CAG ATT CTG GAA TAT TTT GAC CGC ATT GGC
 577  Gly Val Gly Arg Lys Leu Ala Ile Gln Ile Leu Glu Tyr Phe Asp Arg Ile Gly

1783  TTT ACG CGT CGT CGT GGA AAT GAT CAT TTA TTG CGC GAC GCA TTA TTA TTT CCG
 595  Phe Thr Arg Arg Arg Gly Asn Asp His Leu Leu Arg Asp Ala Leu Leu Phe Pro

1837  GAA AAA TAA GGAAATGATT
 613  Glu Lys ***
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FIG. 1 Nucleotide sequence of the *selB* gene and its derived amino-acid sequence. The 2.1 kilobase (kb) insert of plasmid pWL144 (ref. 6) containing the *selB* gene was sequenced by the method of Maxam and Gilbert[16] using either restriction sites within the plasmid or the endpoints of nested Bal31 deletion clones. The putative Shine–Dalgarno sequence is underlined and the termination codon is indicated by asterisks. The sequence data are deposited in the EMBL database under the accession number X16644.

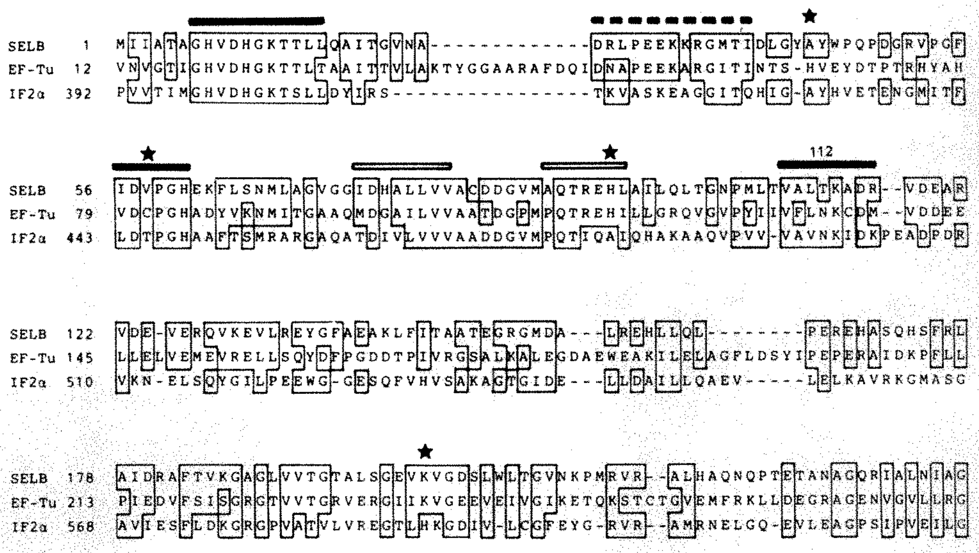

SELB 1 MIIATAGHVDHGKTTLLQAITGVNA-------------DRLPEEKKRGMTIDLGYAYWPQPDGRVPGF
EF-Tu 12 VNVGTIGHVDHGKTTLTAAITTVLAKTYGGAARAFDQIDNAPEEKARGITINTS-HVEYDTPTRHYAH
IF2α 392 PVVTIMGHVDHGKTSLLDYIRS----------------TKVASKEAGGITQHIG-AYHVETENGMITF
112
SELB 56 IDVPGHEKFLSNMLAGVGGIDHALLVVACDDGVMAQTREHLAILQLTGNPMLTVALTKADR--VDEAR
EF-Tu 79 VDCPGHADYVKNMITGAAQMDGAILVVAATDGPMPQTREHILLGRQVGVPYIIVFLNKCDM--VDDEE
IF2α 443 LDTPGHAAFTSMRARGAQATDIVLVVVAADDGVMPQTIQAIQHAKAAQVPVV-VAVNKIDKPEADPDR
SELB 122 VDE-VERQVKEVLREYGFAEAKLFITAATEGRGMDA---LREHLLQL--------PEREHASQHSFRL
EF-Tu 145 LLELVEMEVRELLSQYDFPGDDTPIVRGSALKALEGDAEWEAKILELAGFLDSYIPEPERAIDKPFLL
IF2α 510 VKN-ELSQYGILPEEWG-GESQFVHVSAKAGTGIDE---LLDAILLQAEV-----LELKAVRKGMASG
SELB 178 AIDRAFTVKGAGLVVTGTALSGEVKVGDSLWLTGVNKPMRVR--ALHAQNQPTETANAGQRIALNIAG
EF-Tu 213 PIEDVFSISGRGTVVTGRVERGIIKVGEEVEIVGIKETQKSTCTGVEMFRKLLDEGRAGENVGVLLRG
IF2α 568 AVIESFLDKGRGPVATVLVREGTLHKGDIV-LCGFEYG-RVR--AMRNELGQ-EVLEAGPSIPVEILG

FIG. 2 Comparison of the N-terminal amino-acid sequence of SELB with the amino-acid sequences of translation factors EF-Tu (ref. 17) and IF-2 (ref. 18) from *E. coli* (single-letter amino-acid code). Identical or chemically similar amino acids are boxed, and the following amino acids were considered as similar: D and E; N and Q; K and R; S and T; I, L, M and V; and F, W and Y. Five regions conserved in G proteins[19] are indicated by horizontal bars. The solid bars correspond to regions directly involved in binding GTP as defined by X-ray crystallography for EF-Tu (ref. 10). The dashed line indicates a region in the sequence that is conserved only among elongation factors[19] and is proposed to be involved in GTPase activity[20]. Amino acids probably located near the aminoacyl-tRNA-binding site of EF-Tu as shown by crosslinking and chemical-modification studies[21,22], are indicated by *. The number 112 indicates the position of the highly conserved asparagine residue of G proteins replaced by threonine in the SELB sequence.

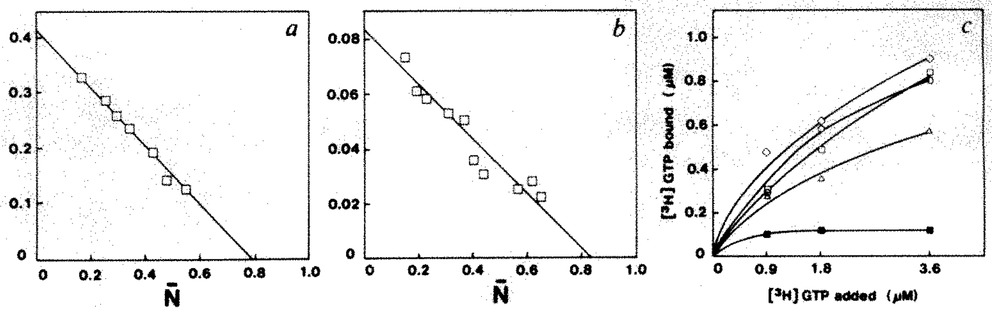
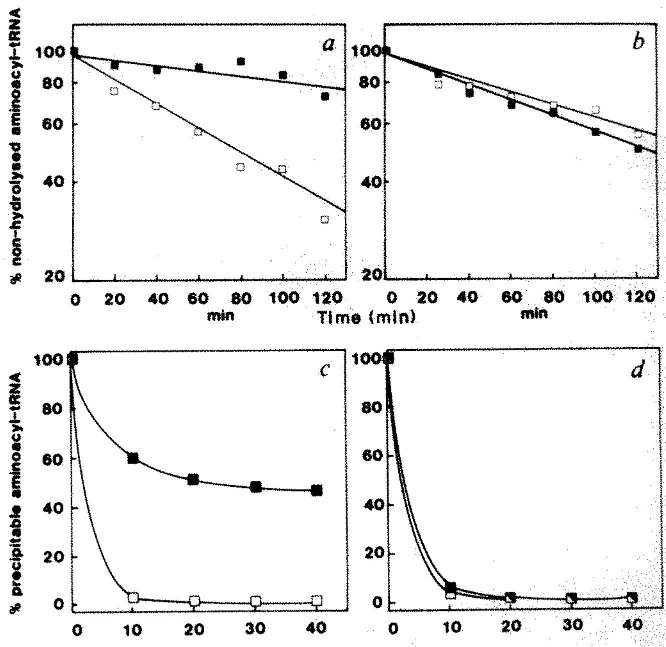


FIG. 3 *a*, *b*, Scatchard plots of GTP (*a*) and GDP (*b*) binding to SELB. $\bar{N}$, Molar concentration of nucleotide bound per mole of SELB. [L], Molar concentration of unbound nucleotide. *c*, Specificity of GTP binding to SELB. A 10-fold excess of unlabelled nucleotide was added to each incubation mixture. □, Without addition; ■, +GTP; ○, +ATP; ◇, +CTP; and △, +UTP.

METHODS. Binding was measured by equilibrium dialysis in 200-μl chambers containing 20 mM potassium phosphate, pH 7.0, 5 mM $MgCl_2$, 100 mM KCl, 1 mM DTT, and 1.3 μM (*a*, *c*) or 4 μM SELB (*b*) and 0.3–4.8 μM [^{3}H]GTP or 2–30 μM [^{3}H]GDP, respectively. After a 16-h incubation at 4 °C, three 50-μl aliquots were taken from each vessel, dried on glass fibre filters, applied to rotoszint scintillation cocktail and counted. The same dissociation constants were obtained when the phosphate buffer was replaced by 50 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, 100 mM KCl, 1 mM DTT.

FIG. 4 Protection of seryl-tRNA^{UCA} (*a*, *c*) and selenocysteyl-tRNA^{UCA} (*b*, *d*) against alkaline hydrolysis (*a*, *b*) or pancreatic RNase digestion (*c*, *d*) in the presence (■) or absence (□) of SELB. *a*, *b*, Hydrolysis protection experiments[13] were carried out in 200 μl of 50 mM potassium phosphate, pH 7.5, 5 mM $MgCl_2$, 100 mM KCl, 0.1 mM cysteine, containing 0.2 μM tRNA^{UCA} aminoacylated with [^{14}C]serine or [^{75}Se]selenocysteine. The reaction mixtures were incubated at 24 °C in the presence or absence of 1 μM SELB. Aliquots (25 μl) were removed and spotted on glass-fibre filters soaked in ice-cold 10% trichloroacetic acid to precipitate the tRNA. The filters were washed 4 times with 2 ml 5% trichloroacetic acid and once with 2 ml 70% ethanol, dried, and their radioactivity determined. *c*, *d*, RNase protection experiments[15] were performed in 100 μl of 20 mM potassium phosphate, pH 7.2, 5 mM $MgCl_2$, 100 mM KCl, 0.1 mM cysteine, containing 0.2 μM tRNA^{UCA} aminoacylated with either [^{14}C]serine or [^{14}C]selenocysteine and in the presence or absence of SELB. Reaction mixtures were incubated for 5 min at room temperature, then cooled on ice. An aliquot of 20 μl was removed and precipitated on glass fibre filters as described above. Then [illegible] μl pancreatic RNase (2 mg ml^{-1}) was added and 20-μl aliquots were removed after 10, 20, 30 and 40 s and the acid-insoluble radioactivity determined as described above. All experiments were carried out in an anaerobic chamber under N_2–H_2 (96:4) atmosphere.



These results strongly indicate that SELB specifically complexes selenocysteyl-tRNA^{UCA} but does not recognize tRNA^{UCA} aminoacylated with serine. Complex formation is independent of the presence of guanine nucleotides, as is complex formation with *E. coli* IF-2 (ref. 14) or EF-Tu from thermophilic bacteria (M. Sprinzl, personal communication). The basis for this specificity towards selenocysteine remains to be elucidated, but could depend on either the tRNA^{UCA} undergoing a conformational change when serine is converted to selenocysteine, or, more plausibly, the direct recognition by SELB of the selenol group at the aminoacyl residue.

SELB most probably functions biochemically as an alternative elongation factor to EF-Tu, specific for the insertion of selenocysteine directed by the UGA codon in the *fdhF* mRNA. Apart from the sequence homology with EF-Tu and IF-2, several other lines of evidence support this role of SELB. First, SELB binds guanine nucleotides and selenocysteyl-tRNA. Second, EF-Tu exhibits a 200-fold lower affinity for seryl-tRNA^{UCA} than for any other aminoacyl-tRNA involved in elongation (G. Ott and M. Sprinzl, personal communication). Furthermore, conversion of the serine residue to a selenocysteine residue does not change this affinity (G. Ott, K.F. and M. Sprinzl, unpublished results). Thus, under *in vivo* conditions, selenocysteyl-tRNA cannot compete for ternary complex formation with EF-Tu. Finally, lesions in *selB* do not interfere with selenocysteyl-tRNA formation[8]; rather, they promote an accumulation of selenocysteyl-tRNA and, despite the resulting intracellular selenocysteyl-tRNA concentration, there is no incorporation of selenocysteine at the specific UGA codon by EF-Tu.

These findings are therefore consistent with SELB being a new G protein functioning as a translation factor that specifically recognizes the aminoacyl residue of a tRNA. The recognition of the selenocysteyl residue esterified to tRNA^{UCA} thus could be the biochemical basis for the extreme specificity with which the UGA codon in the *fdhF* mRNA directs the insertion of selenocysteine only, and not the insertion of L-serine; this is exemplified in mutants that cannot convert seryl-tRNA^{UCA} into selenocysteyl-tRNA[8]. The higher M_r of SELB (68K) relative to that of EF-Tu (43K) indicates that SELB probably has additional functions. One function of SELB could be to recognize the aminoacyl residue. Other functions could be the specific recognization of the mRNA context around the selenocysteine-specific UGA codon and/or to compete for the binding of release factor-2. □

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1. Zinoni, F., Birkmann, A., Leinfelder, W. & Böck, A. *Proc. natn. Acad. Sci. U.S.A.* **84,** 3156–3160 (1987).
2. Böck, A. & Stadtman, T. C. *Biofactors* **1,** 245–250 (1988).
3. Zinoni, F., Birkmann, A., Stadtman, T. C. & Böck, A. *Proc. natn. Acad. Sci. U.S.A.* **83,** 4650–4654 (1986).
4. Chambers, I. *et al. EMBO J.* **5,** 1221–1227 (1986).
5. Sukenaga, Y., Ishida, K., Takeda, T. & Takagi, K. *Nucleic Acids Res.* **15,** 7187 (1987).
6. Leinfelder, W. *et al. J. Bacteriol.* **170,** 540–546 (1988).
7. Leinfelder, W., Zehelein, E., Mandrand-Berthelot, M. A. & Böck, A. *Nature* **331,** 723–725 (1988).
8. Leinfelder, W., Stadtman, T. C. & Böck, A. *J. biol. Chem.* **264,** 9720–9723 (1989).
9. Dever, T. E., Glynias, M. J. & Merrick, W. C. *Proc. natn. Acad. Sci. U.S.A.* **84,** 1814–1818 (1987).
10. LaCour, T. M. F., Nyborg, J., Thirpu, S. & Clark, B. F. C. *EMBO J.* **4,** 2385–2388 (1985).
11. Gualerzi, C. O. *et al.* in *Structure, Function, and Genetics of Ribosomes* (eds Hardesty, B. & Kramer, G.) 621–641 (Springer, New York, 1986).
12. Arai, K., Kawakita, M. & Kaziro, Y. *J. Biochem., Tokyo* **76,** 293–306 (1976).
13. Pingoud, A., Urbanke, C., Krauss, G., Peters, F. & Maass, G. *Eur. J. Biochem.* **78,** 403–409 (1977).
14. Petersen, H. U., Roll, T., Grunberg-Manago, M. & Clark, B. F. C. *Biochem. biophys. Res. Commun.* **91,** 1068–1074 (1979).
15. Knowlton, R. G. & Yarus, M. *J. Molec. Biol.* **139,** 721–732 (1980).
16. Maxam, A. M. & Gilbert, W. *Meth. Enzym.* **65,** 499–561 (1980).
17. Jones, M. D. *et al. Eur. J. Biochem.* **108,** 507–526 (1980).
18. Sacerdot, C. P., Dessen, P., Hershey, J. W. B., Plumbridge, J. A. & Grunberg-Manago, M. *Proc. natn. Acad. Sci. U.S.A.* **81,** 7787–7791 (1984).
19. Kohno, K. *et al. Proc. natn. Acad. Sci. U.S.A.* **83,** 4978–4982 (1986).
20. Van Noort, J. M. *et al. Eur. J. Biochem.* **160,** 557–561 (1986).
21. Jonak, J., Pokorna, K., Meloun, B. & Karas, K. *J. Biochem.* **154,** 355–362 (1986).
22. Van Noort, J. M., Kraal, B. & Bosch, L. *Proc. natn. Acad. Sci. U.S.A.* **82,** 3212–3216 (1985).
23. Leinfelder, W., Forchhammer, K., Veprek, B., Zehelein, E. & Böck, A. *Proc. natn. Acad. Sci. U.S.A.* (in the press).

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The conformation of the DNA double helix in the crystal is dependent on its environment

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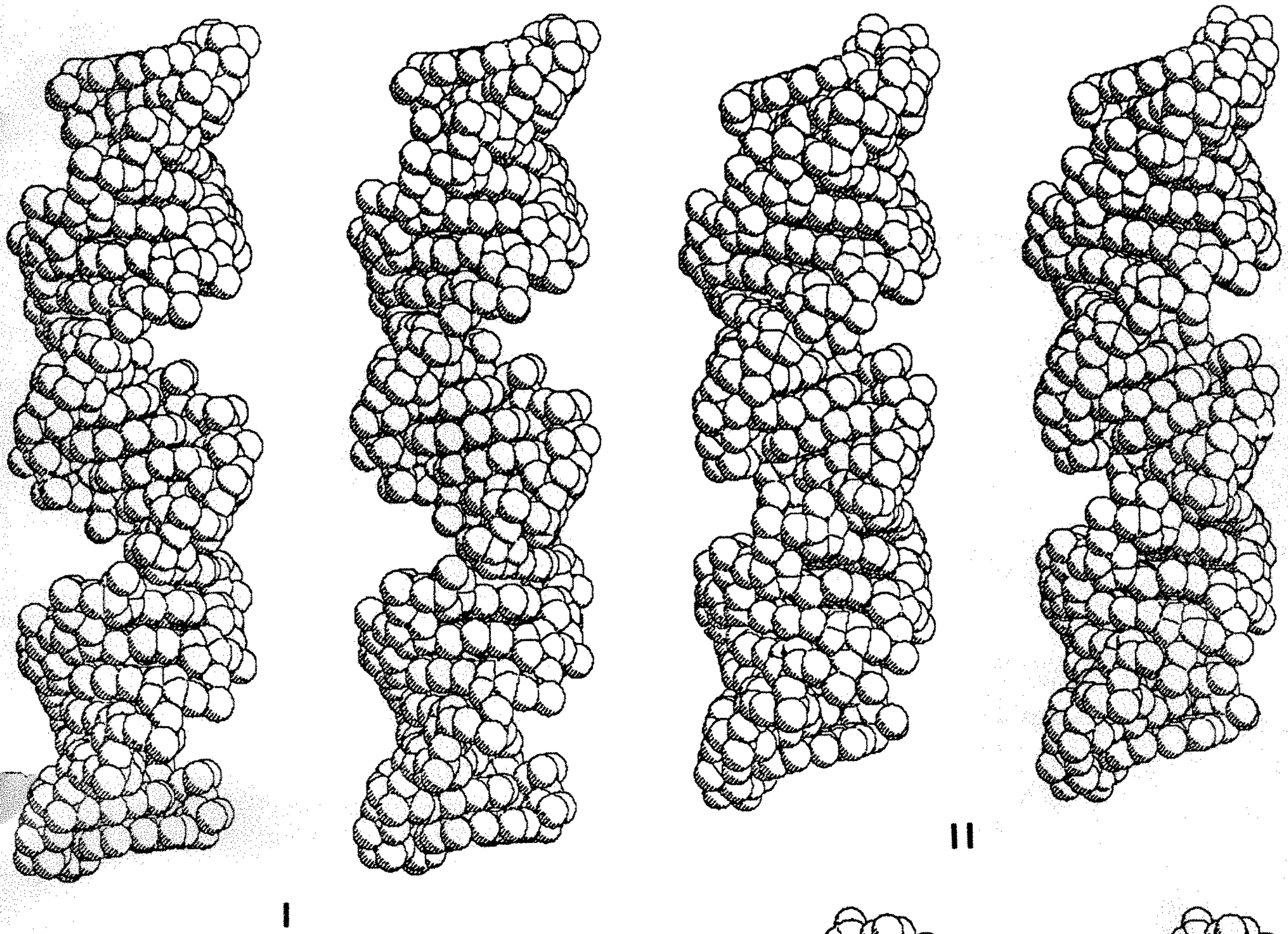
STUDIES of the crystal structures of more than 30 synthetic DNA fragments have provided structural information about three basi forms of the double helix: A-, B- and Z-form DNA[1–5]. Thes studies have demonstrated that the DNA double helix adopts highly variable structure which is related to its base sequence. The extent to which such observed structures are influenced by the crystalline environment can be found by studying the same molecule in different crystalline forms. We have recently crystallized one particular oligomer in various crystal forms. Here we report the results of structural analyses of the different crystal structures and demonstrate that the DNA double helix can adopt a range of conformations in the crystalline state depending on hydration, molecular packing and temperature. These results have implications on our understanding of the influence of the environment on DNA structure, and on the modes of DNA recognition by proteins.

The octamer d(G-G-G-C-G-C-C-C) or G-G-G-C-G-C-C-C has been crystallized as an A-DNA double helix in two crystal forms, tetragonal and hexagonal. The structure of each form was determined at room temperature (RT) and low temperature (LT). The experimental details for crystallization, data collection and structure determination are given in Table 1.

The crystal structures of the tetragonal forms have been previously reported[6,7] and their double-helical structures at the two temperatures found to be very similar; therefore, we refer to only the RT structure of the tetragonal form. We found that when hexagonal crystals are cooled, however, there are drastic changes in unit-cell volume and resolution of the data (Table 1). Such changes indicate a phenomenon of phase transition, and therefore the crystalline environment of the hexagonal LT structure is different from that of the RT structure. Some of the global and local parameters of each of the three structures of the same sequence are given in Table 2, and continuous helices based on the three structures displayed in Fig. 1. Each of these three crystal structures corresponds to a distinct conformation of the A-DNA double helix. The tetragonal structure is of conformation I. This is characterized by a very small tilting (inclination) of the base pairs with respect to the overall helix axis, a large rise per base-pair and a widely open major groove. The hexagonal RT structure is of conformation II, with medium values of base tilting and rise. The major groove is narrower and deeper than that of the tetragonal structure. The hexagonal LT structure is of conformation III. This is a slim helix with a narrower and shallower major groove than those of conformations I and II. The tilting of the bases and the rise are similar to those of conformation II.

These data show that the same oligomer can assume more than one conformation in the crystalline state. The three helices are embedded in three distinct crystalline environments. Among the factors that are likely to influence molecular conformation and crystal packing are hydration and temperature. Analysis of the water structure in the three crystals shows that the level and mode of hydration changes considerably from one conformational type to the next. Conformation I is highly hydrated, II is poorly hydrated and III is fairly hydrated. It seems that during the cooling process of the hexagonal crystals their water structure undergoes an important rearrangement which affects both the helical conformation and packing arrangement.



It is reasonable to assume that during the process of crystallization, the double-helical conformation changes continuously depending on water activity, concentration of counter ions and alcohol. When crystal nucleation is initiated at a state of relatively high water activity then the tetragonal form appears, whereas at low-water activity the hexagonal form crystallizes out. This is also consistent with the crystallization experiments (Table 1). Readjustment of the overall helical shape can also occur during crystal formation to optimize intermolecular interactions. An important observation is that all previously determined A-type octamers crystallized in either tetragonal or hexagonal forms[4,5]. Those of the tetragonal space group were shown to adopt a common elongated helical shape similar to conformation I, whereas most of the ones crystallizing in the hexagonal space group were stubby and short helices similar to conformation II.

The modes of intermolecular interactions observed in the two crystal forms have been extensively studied[6,8-11]. All these interactions involve the van der Waals contact of a terminal base pair with the flat minor groove of an adjacent duplex. In some cases the intermolecular contact is further stabilized by hydrogen bonds depending on its location at the minor groove and the particular base sequence. We have not found any substantial evidence to show that the difference in global structure observed in the different crystalline environments is caused by such interactions. But we do not rule out the possibility that water-mediated intermolecular contacts may be important in this respect.

Thus, we argue that either packing requirements control the overall helical shape or a particular conformation crystallizes in the appropriate crystal form. In any event, the important finding is that the observed structure and crystal packing are mutually dependent.

What is the role of the base sequence in determining the conformation of DNA in the crystalline state? Previous studies

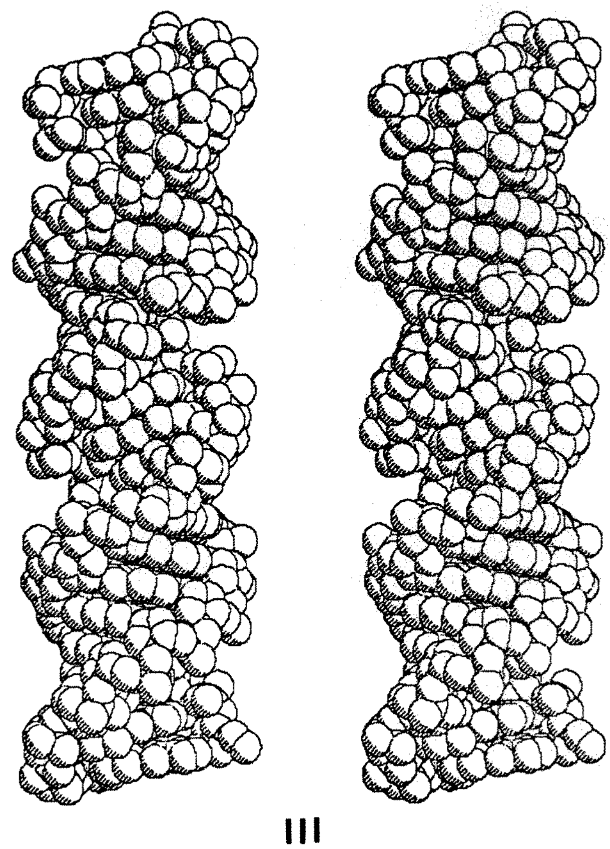


FIG. 1 Stereoscopic space-filling drawings of continuous helices based on conformations I, II and III. Each helix (24 base pairs long) was generated by rotating and translating the corresponding double-helical octamer with respect to the overall helix axis, using the average values of helix twist and rise per base pair of Table 2.

have demonstrated a correlation between the local structure and the base sequence. The effect of the base sequence in A- and B-DNA is shown primarily by variations in base-stacking geometry and, to a much lesser extent, by changes in the sugar-phosphate backbone[4,5]. Variations in the arrangement of the base pairs can be estimated from the relative rotations and translations of adjacent base pairs. The following three of these parameters are largely variable in the crystal structures: (1) the local helix twist, which is the relative rotation of two successive base pairs with respect to the helix axis; (2) the roll angle, a measure of the opening of the base pairs towards the helical grooves; and (3) the slide, which is the relative displacement of the base pairs along their long axes. The values of these parameters for each of the three structures are given in Table 2. Significant changes are observed from one crystal to the other.

The central pyrimidine-purine site of the sequence used in the study described here has been studied in several other A-type octamers: C-C-C-C-G-G-G-G (ref. 9), G-G-C-C-G-G-C-C (ref. 10, 11), and G-C-C-C-G-G-G-C (ref. 12). In all these structures as well as the tetragonal form of G-G-G-C-G-C-C-C (conformation I), the C-G step adopts a unique stacking geometry with a small twist (average 24°) and large negative slide (average −2 Å). The roll angles of the C-G doublets display a wide range of values (0–12°). The sugar-phosphate backbone of each of the C-G sites adopts an extended structure in contrast to the usual folded conformation observed in all other sites.

Only one of the three common properties is retained in the C-G step of conformation II, namely the helix twist (24°). The amount of slide is smaller (−1.3 Å). The values of the two parameters in conformation III (29° and −1.0 Å) differ much more from those of conformation I. The central backbones of conformations II and III have the usual folded structure. The stacking geometries of the C-G doublets in conformations I, II and III are shown in Fig. 2. The change in sliding motion is reflected by the amount of overlapping between the centr guanine bases. The overlapping diminishes gradually going from conformation I by way of II to III. The decrease in interstrand stacking between the guanines is associated with a simultaneous increase in intrastrand stacking between the guanine and cytosine bases.

The various parameters of several other helical steps also differ significantly between the different crystals. We believe that such local variations are not induced directly by crystal-packing forces for the following reasons. As mentioned before, the intermolecular interactions in all crystal forms are relatively soft and non-specific. Moreover, the 2-fold symmetry is largely retained in the duplexes that do not incorporate crystallographic symmetry axes[1,4]. Even in cases where intermolecular interactions deform asymmetrically the double helix as for the B-dodecamer series[2], the local structure of equivalent base pairs is similar.

We suggest that changes in local structure of the same molecule in different crystalline environments are induced by hydration effects. This is supported by a common water structure observed in all C-G sites displaying the specific geometry described above (Fig. 3), in which a string of water molecules runs across the minor groove and interacts directly with the two guanines and two 3′-phosphate groups. This water structure is further stabilized by four water branches which are hydrogen-bonded to the sugar-phosphate backbone of two nearby symmetry-related duplexes. Any sliding of the base pairs in a direction that reduces the overlap of the guanines would disrupt this

TABLE 1 X-ray data of d(G-G-G-C-G-C-C-C)

	I	II	III
Temperature	293 K	293 K	100 K
Space group	$P4_32_12$	$P6_1$	$P6_1$
a (Å)	43.28	46.96	43.62
b (Å)	43.28	46.96	43.62
c (Å)	24.66	44.31	41.49
V (Å^3)	46,192	84,623	68,367
No. of oligomers	8	12	12
Resolution (Å)	1.7	2.9	1.9
No. of reflections at 2σ level	2,077	1,008	2,827
R-factor (%)	16.2	12.0	16.0
No. of ordered waters per duplex*	88	45	96

The deoxyoligonucleotide was prepared by the solid-phase procedure using fast phosphoramidite chemistry[15], and purified by ion-exchange and HPLC. Crystallization was done at 19 °C using solutions containing 2 mM DNA, 5 mM $MgCl_2$, 0.15 mM spermine–HCl, 5% 2-methyl-2,4-pentanediol (MPD) and 50 mM sodium cacodylate (pH 7.0), equilibrated against reservoirs of concentrated MPD solutions (30 or 50%). Tetragonal crystals appeared predominantly at 30% MPD whereas hexagonal crystals appeared at 50% MPD when the drops almost dried out. The hexagonal crystals could be preserved only by adding buffered solutions containing 75% MPD. Diffraction data were measured at both RT and LT on a Rigaku AFC5 diffractometer equipped with a rotating anode source. For the RT experiment the crystal was sealed in a glass capillary containing a droplet of mother liquor. For the LT experiment a naked crystal was mounted directly and shock-cooled to 100 K (ref. 16). The structures were solved by the multi-dimensional search method ULTIMA[17] and refined using the restrained least-squares program by Hendrickson and Konnert[18]. Solvent peaks located from electron density maps and satisfying hydrogen-bonding criterions were included in the refinement as oxygen atoms.

* Although the total number of ordered waters included in the refinement of conformation I was smaller than that of conformation III, the number of waters located at the first and second hydration shells of conformation I was 20 more than that for conformation III.

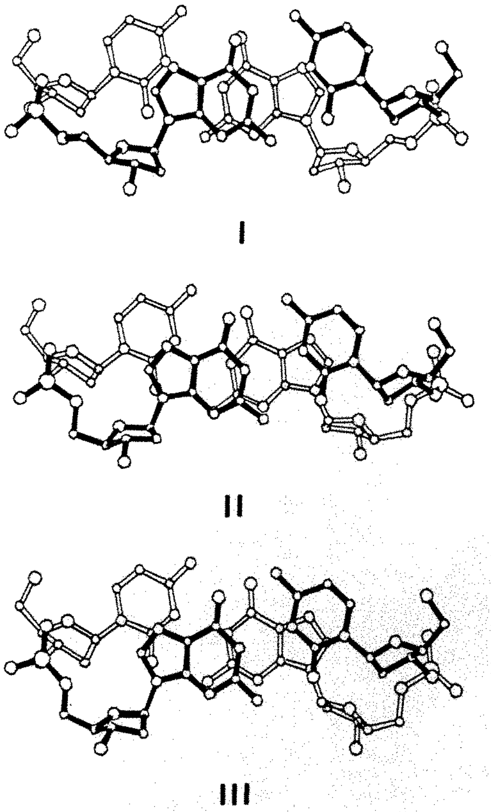


FIG. 2 Stacking geometry of C-G doublets in conformations I, II and III. The view is perpendicular to the upper base pair. It shows the decrease in slide from conformation I by way of II to III, and the increase in helix twist from conformations I and II to III.

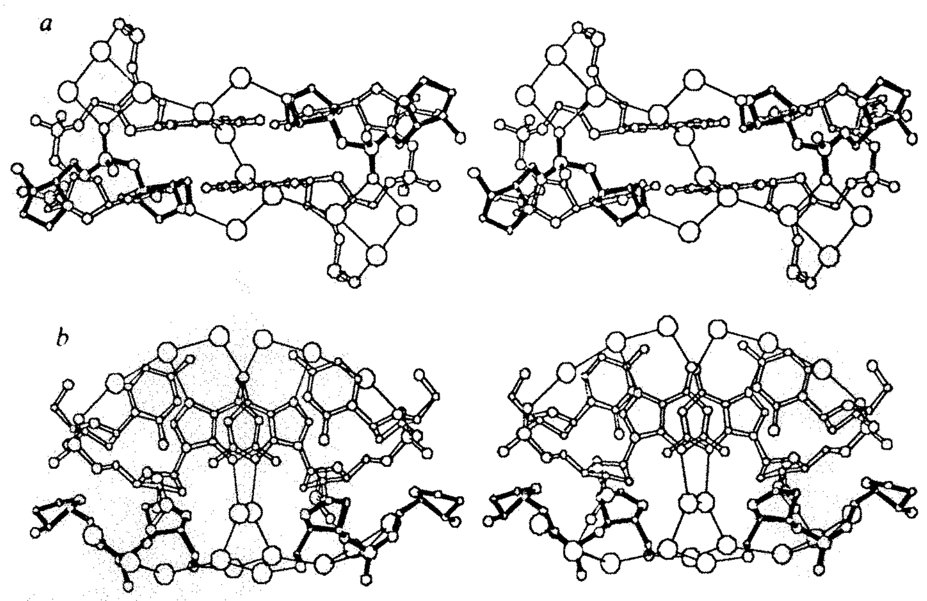


FIG. 3 Stereoscopic drawings of hydration of the C-G doublet in conformation I. *a*, The view is down the minor groove showing the interactions of water molecules with N3 atoms of guanines and the adjacent 3′-phosphate oxygens. The water structure is stabilized by hydrogen bonds to the sugar-phosphate backbone of G1 and G2 from neighbouring duplexes (dark bonds). *b*, The view is perpendicular to the upper base pair, showing the hydration at both grooves. At the major groove a string of waters is hydrogen bonded to O6, N7 and 5′-phosphate oxygen atoms of each of the guanine residues.

Hydration. At the major groove, strings of water molecules interact with the guanine bases and the 5′-phosphate oxygens of the locally extended backbone. Hence, the unique local structure observed in all the tetragonal C-G sites is stabilized by this water structure. An equivalent type of hydration cannot be accommodated in the hexagonal crystals because part of the central region of the minor groove is blocked by a neighbouring molecule, thus demonstrating the indirect influence of crystal packing on local DNA structure.

TABLE 2 Helix parameters

Structure	Global parameters I	II	III
Helix twist (°)	32 (4)	31 (5)	32 (2)
Rise per base-pair (Å)	3.3 (0.1)	2.9 (0.3)	2.9 (0.3)
Base-pair inclination (°)	7 (1)	13 (2)	14 (1)
Propeller twist (°)	−9 (3)	−6 (6)	−9 (5)
Base-pair displacement dx (Å)	−3.8 (0.2)	−4.9 (0.3)	−3.5 (0.2)
Helix diameter (Å)	19.5 (0.9)	19.7 (0.8)	18.1 (0.4)
Major-groove width (Å)	10.9 (0.7)	8.0 (0.4)	5.6 (0.5)
Minor-groove width (Å)	9.4 (0.3)	9.7 (0.6)	10.3 (0.5)

Local parameters

Helical step	Twist (°) I	II	III	Roll (°) I	II	III	Slide (Å) I	II	III
G-G	32.3	29.4	32.0	7.2	1.2	3.7	−1.4	−2.2	−1.6
G-G	31.2	28.9	36.0	6.4	13.2	5.5	−1.7	−2.2	−1.3
G-C	34.7	35.5	32.6	3.7	2.0	7.1	−1.3	−1.6	−0.7
C-G	24.0	23.7	29.0	3.5	8.9	10.0	−2.2	−1.3	−1.0
G-C	34.7	37.3	32.0	3.7	3.5	6.1	−1.3	−2.0	−0.8
C-C	31.2	29.9	33.4	6.4	10.3	7.5	−1.7	−2.4	−1.7
C-C	32.3	30.0	30.8	7.2	11.1	6.9	−1.4	−1.5	−1.5
Average	31.5	30.6	32.3	5.4	7.2	6.7	−1.6	−1.9	−1.2
s.d.	3.6	4.5	2.2	1.7	4.9	1.9	0.3	0.4	0.6

The numbering of residues from 5′ to 3′ is G1 to C8 in the first strand and G9 to C16 in the second. Hence, the first G-G step consists of base pairs G1·C16 and G2·C15, and so on. Helix diameter was calculated from the average distance of phosphorus atoms to the overall helix axis. The groove widths were estimated from averaging the shortest P–P distances across each groove of the continuous helix. Definitions of the other parameters are given in refs 2 and 19. The convention of signs used here is the one agreed on at an EMBO workshop on DNA curvature and bending[20]. Hence, propeller twist, base-pair displacement and slide are negative unlike those of previous publications. The s.d. (given in parentheses) is the r.m.s. deviation from the mean. I, Tetragonal conformation; II, hexagonal RT conformation; III, hexagonal LT conformation.

The results presented here have implications on the modes of DNA recognition by proteins[13,14]. The specificity of the recognition of a sequence of bases and hence the stability of the DNA-protein interface are dependent on the correct alignment of the base pairs along the helix. We show that a specific alignment of two successive base pairs is driven by a local hydration. It is therefore reasonable to anticipate that even larger changes in the orientation and position of the DNA base pairs can be accomplished by direct or water-mediated interactions with the protein. Also, specific hydration of the base pairs at one groove may govern the degree of helical twist, roll and slide needed to accommodate protein–DNA contacts at the other groove.

From the results of this study we conclude that the DNA double helix can adopt a range of conformations in the crystalline state depending on hydration, molecular packing and temperature. Such studies demonstrate the capability of DNA to undergo important conformational changes as a result of modest variations in its environment. □

Note added in proof. Two conformations in the crystal were recently reported for another A-DNA octamer[21].

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1. Shakked, Z. & Kennard, O. in *Biological Macromolecules and Assemblies: Nucleic Acids and Interactive Proteins* Vol. 2 (eds Jurnak, F. A. & McPherson, A.) 1–36. (Wiley, New York, 1985).
2. Dickerson, R. E., Kopka, K. L. & Pjura, P. in *Biological Macromolecules and Assemblies: Nucleic Acids and Interactive Proteins* Vol. 2 (eds Jurnak, F. A. & McPherson, A.) 38–126 (Wiley, New York, 1985).
3. Wang, A. H.J-. & Rich, A. in *Biological Macromolecules and Assemblies: Nucleic Acids and Interactive Proteins* Vol. 2 (eds Jurnak, F. A. & McPherson, A.) 128–170 (Wiley, New York, 1985).
4. Shakked, Z. & Rabinovich, D. *Progr. Biophys. molec. Biol.* **47,** 159–195 (1986).
5. Haran, T. E. & Shakked, Z. *J. molec. Struct.* **179,** 367–391 (1988).
6. Rabinovich, D., Haran, T. E., Eisenstein, M. & Shakked, Z. *J. molec. Biol.* **200,** 151–161 (1988).
7. Eisenstein, M. *et al. Acta crystallogr.* **B44,** 625–628 (1988).
8. Shakked, Z. *et al. Proc. R. Soc.* **B213,** 479–487 (1981).
9. Haran, T. E., Shakked, Z., Wang, A., & Rich, A. *J. biomolec. Struct. Dynam.* **5,** 199–217 (1987).
10. Wang, A. H.-J., Fujii, S., van Boom, J. H. & Rich, A. *Proc. natn. Acad. Sci. U.S.A.* **79,** 3968–3972 (1982).
11. Haran, T. E. thesis, Feinberg Grad. Sch. Weizmann Inst. Sci. (1986).
12. Heinemann, U., Lauble, H., Frank, R. & Blocker, H. *Nucleic Acids Res.*
13. von Hippel, P. H. & Berg, O. G. *Proc. natn. Acad. Sci. U.S.A.* **83,** 1608–1612 (1986).
14. Otwinowski, Z. *et al. Nature* **335,** 321–329 (1988).
15. Beaucage, S. L. & Caruthers, M. H. *Tetrahedron Lett.* **2,** 1859–1862 (1981).
16. Hope, H. *Acta Crystallogr.* **B44,** 22–26 (1988).
17. Rabinovich, D. & Shakked, Z. *Acta crystallogr.* **A40,** 195–200 (1984).

18. Hendrickson, W. A. & Konnert, J. H. in *Biomolecular Structure, Conformation, Function and Evolution* Vol. 1 (eds Srinivasan, R. Subramanian, E. & Yatindra, N.) 43–57 (Pergamon, Oxford, 1981).
19. Fratini, A. V., Kopka, M. L., Drew, H. R. & Dickerson, R. E. *J. biol. Chem.* **257**, 14686–14707 (1982).
20. Dickerson, R. E. *et al. EMBO J.* **8**, 1–4 (1989).
21. Jain, S. & Sundaralingam, M. J. *J. biol. Chem.* **264**, 12780–12784 (1989).

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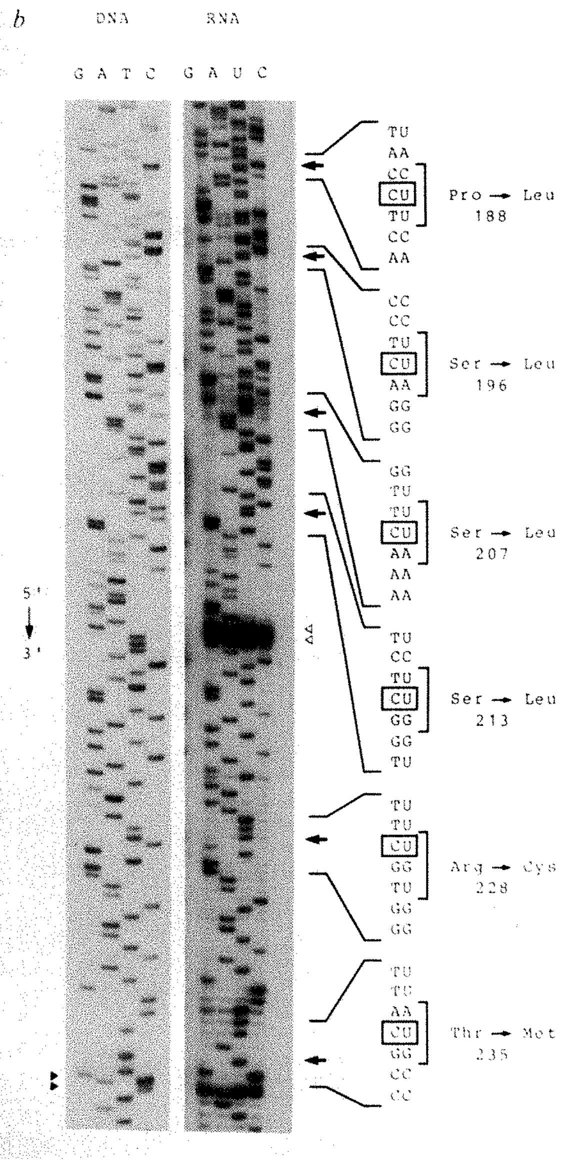
ERRATUM

RNA editing in plant mitochondria

Patrick S. Covello & Michael W. Gray

Nature **341**, 662–666 (1989)

FIGURE 2*b* in this letter was printed incorrectly, so that the gels were rotated through 180° and do not correspond to the labels. The correct version is printed below and will appear in reprints.



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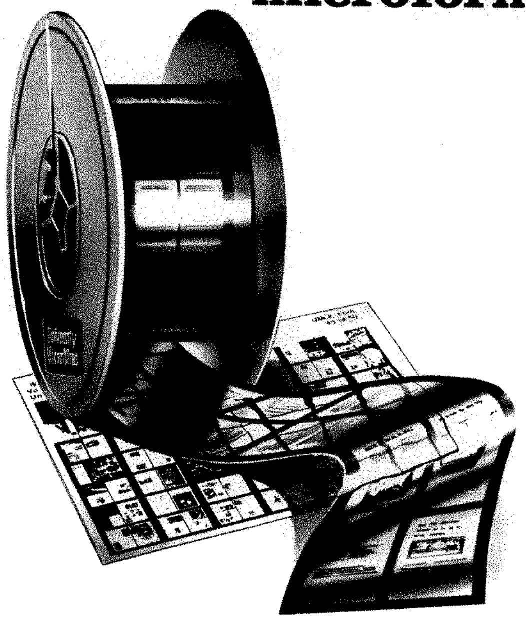

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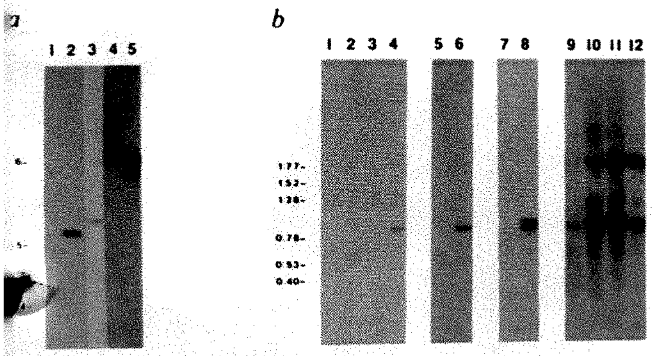
ion of a functioning cutinase gene, we inserted this gene from F. *solani pisi* into *Mycosphaerella* spp. by a transformation procedure using whole fungal cells and lithium acetate treatment[4,8]. The plasmid pU5-11 contains the cutinase structural gene and nearly 4 kilobases (kb) of 5′ upstream sequence, plus kb of 3′ non-coding DNA[4]. *Mycosphaerella* was cotransformed with this plasmid and a plasmid containing the hygromycin-resistance gene[4,9,10]; the transformants were selected for hygromycin resistance and screened for the cutinase gene by Southern blot analysis (Fig. 1). The four transformants shown in Fig. 1 were cut with *Sty*I, an enzyme that does not cut within pU5-11. The integration events seem to be random. The transformants M-25 and M-101 (lanes 4 and 5) probably contain tandem duplications. Because signals of high molecular weight were present, it is likely that M-101 also has another separate site of integration.

We grew the transformants under inducing conditions[11] to ee whether the cutinase gene that is induced by cutin monomers in *F. solani pisi*[12] is transcriptionally regulated in the *Mycosphaerella* transformants as well. Northern blots of transcripts rom wild type and the four *Mycosphaerella* transformants were hybridized with radioactively labelled cutinase cDNA from *Fusarium* as probe (Fig. 1). An interval of 48 hours was chosen because in that time the level of transcription of the gene in *Fusarium solani pisi* reaches a maximum[13]. There was no hybridization with the wild-type *Mycosphaerella* RNA (lanes 1 and 2), showing that transcripts from this organism share no homology with *Fusarium* cutinase messenger RNA. Transformants M-12 (lane 4) and M-21 (lane 6) displayed similar patterns, with a unique transcript comparable in size to authentic *Fusarium* cutinase mRNA. Transformants M-25 and M-101 gave wo and three separate transcripts, respectively. These multiple transcripts might have been due to differential processing or splicing of the mRNA; alternatively, insertion in different positions could lead to the production of transcripts of different sizes in *Mycosphaerella*. A time course of induction of cutinase gene in transformant M-101 (Fig. 1, lanes 8-12) showed a low level of constitutive expression of the gene and maximal expression at 48 h after the addition of the inducer.

To investigate whether the transformants that produced transcripts containing the coding sequence for cutinase were translated, the culture supernatants from the induced cultures were subjected to western blot analysis, using as probe antibodies raised against *Fusarium solani pisi* cutinase. There was no cutinase detectable from the wild type even after 72 h of induction (Fig. 2). All four transformants produced cutinase within 72 h of addition of cutin hydrolysate. Within 24 h of adding inducer, the transformant M-101 was producing cutinase, which increased up to 72 h; For transformant M-25, cutinase levels were fairly high by 72 h, reaching 74% of the M-101 cutinase levels, although the induction was slower (data not shown). Transformants M-21 and M-12 yielded much less cutinase and this could only be found 72 h after the addition of the inducer.

Next we tested the proteins that were generated by the transformants and which cross-reacted with antibodies against *Fusarium* cutinase to see whether they showed enzyme activity. Cutinase activity correlated with the levels of the proteins detected by immunoblot analyses (Fig. 2): transformant M-101 gave the highest cutinase activity, followed by M-25, whereas supernatants from M-21 and M-12 were much less active. Thus, the levels of transcription, translation product and cutinase activity from the transformants were all in agreement.

We used a papaya fruit bioassay[5] to investigate the pathogenicity of *Mycosphaerella* transformants expressing the inducible cutinase gene. Both wild-type *Mycosphaerella* and the transfor-



IG. 1 *a*, Southern blot analysis of genomic DNA from wild-type *Mycosphaerella* spp. and transformants. Lane 1, wild type; lanes 2, 3, 4 and 5 epresent transformants M-12, M-21, M-25 and M-101, respectively. Co-transformation was as described[4], with 10 μg of each plasmid yielding 2–4 stable transformants per μg; about half of these showed mitotic stability and were analysed by Southern blotting. Genomic DNA from these transformants was digested with *Sty*I, electrophoresed in 0.7% agarose, transferred to nitrocellulose and probed with nick-translated ^{32}P-labelled cDNA for cutinase from *F. solani pisi*[3]. Sizes in kilobases and migration of lambda *Hind*III–*Eco*RI fragments are shown on the left. *b*, Northern blots showing induced cutinase transcript levels in the transformants of *Mycosphaerella* spp. Lanes 1 and 2, wild-type *Mycosphaerella* uninduced and induced for 48 h respectively; lanes 3 and 4, 5 and 6, 7 and 8 represent transformants M-12, M-21, M-25 respectively: in each case the odd number is uninduced and the even number is induced for 48 h; lanes 9, 10, 11, and 12 represent transformant M-101 induced for 0, 24, 48 and 72 h, respectively. In each case glucose-grown fungi were induced with cutin hydrolysate (10 mg ml^{-1}) and RNA isolated with guanidium hydrochloride[16] was separated on 1% formaldehyde agarose gels, transferred to nylon and hybridized to ^{32}P-labelled cutinase cDNA, as described in the legend to Fig. 1*a*. The total RNA levels loaded in each lane did not vary, as we confirmed by washing off the cutinase probe and reprobing the blot with ^{32}P-labelled actin cDNA.

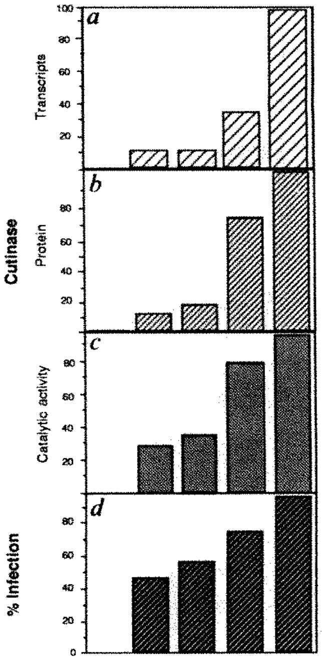


FIG. 2 Correlation of levels of cutinase transcripts (*a*), protein (*b*), enzymatic activity (*c*), with infectivity (*d*) of transformants of *Mycosphaerella* spp. The level of cutinase transcripts was measured by scanning the autoradiograph of the northern blot corresponding to a 48-hour period of induction, as described in the legend of Fig. 1*b*; the levels of cutinase protein produced were measured by scanning the western blot of culture supernatants from 72 h-induced cultures; the levels of enzymatic activity of cutinase in the culture supernatants were determined using *p*-nitrophenylbutyrate as the substrate[11] after 72 h induction; the per cent infection of the wild type and the transformants on intact papaya fruit was measured as described[5]. In all cases, data were normalized against values for transformant M-101 taken as 100. The cutinase activity and per cent infection for M-101 were 43.7 nmol min^{-1} ml^{-1} and 90%, respectively.

mants cause lesions when inoculated on wounds (data not shown). The wild type did not cause lesions when inoculated on intact fruits without wounds (Fig. 3), but the transformants expressing the *Fusarium* cutinase gene did. Frequency and size of lesions were the largest for transformant M-101; nearly all locations inoculated were infected. In fact, the incidence of infection and the severity of the lesions caused by this transformant were essentially identical to those observed with *Colletotrichum gloeosporioides*, an authentic pathogen of intact papaya fruits[5]. The extent to which the transformants become parasites on papaya fruit therefore correlates extremely well with the expression of *Fusarium* cutinase gene (Fig. 2).

To test whether infection of intact fruits by the transformant was due to cutinase, rabbit antibodies against the cutinase from *F. solani pisi* were included with the spore suspension of the M-101 transformant, which produced the most cutinase and the most severe lesion. This antibody preparation, which inhibits *Fusarium* cutinase, prevented lesion formation by transformant M-101 (Fig. 3). Thus, it is clear that *Fusarium* cutinase activity produced by the *Mycosphaerella* transformant enabled it to penetrate the intact fruit and infect it.

We have shown that by inserting a gene encoding a cuticle-degrading enzyme from a pathogenic organism into one that infects only through wounds, the latter can now infect intact plant organs without a wound. As *Mycosphaerella* spp. is able to infect wounded tissue, then this fungus must have most of the genetic machinery required for parasitism. Apparently, however, it cannot penetrate the host's first line of physical defence, the cuticle. Overcoming this limitation by gene insertion allows this organism to infect intact papaya fruits.

Regulation of the *Fusarium* cutinase gene is unchanged when inserted into *Mycosphaerella*: on contact of the spore with the plant surface, gene expression is triggered by cutin monomers released by the small amount of cutinase carried by the spore. The transformants that efficiently expressed cutinase showed low levels of constitutive expression (Fig. 1, lane 9; als confirmed by Western blot analysis, data not shown). The resulting level of cutinase would release monomers from papaya cuti Dihydroxyhexadecanoic acid is a most effective inducer of t cutinase gene in *Fusarium*[7,12] and is the most abundant monom of papaya cutin[14], so the appropriate inducer is available to th transformants when placed on the fruit surface, enabling the to use their regulated expression of cutinase to gain entry int the fruits and infect them. Activation of the cutinase gene i isolated nuclei from *F. solani pisi* by the unique cutin monome has been demonstrated[12]. This selective initiation of transcription of the cutinase gene required a protein factor from th *Fusarium* extract. Apparently *Mycosphaerella* spp. possesses thi protein or a functional analogue, as it can use cutin hydrolysat to activate the *Fusarium* cutinase gene in a similar way. This i not surprising, as similar target proteins that regulate transcription are present in many eukaryotes[15]. By understanding th regulation of genes essential for pathogenesis, it might b possible to interfere with their expression and hence pathogenesis. □

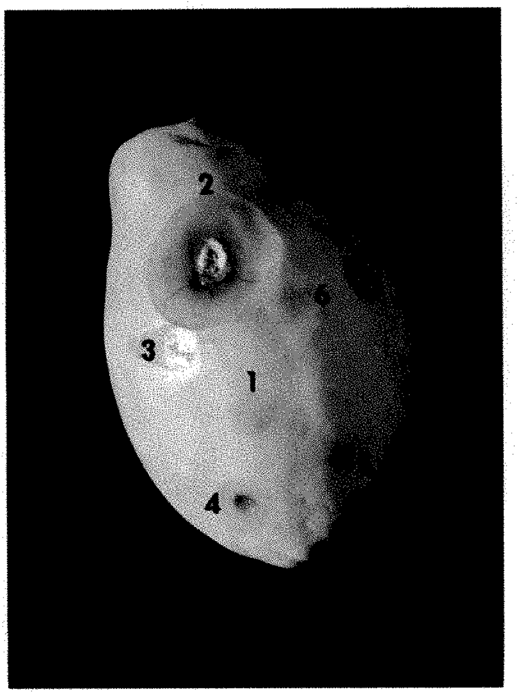


FIG. 3 Lesion formation on intact papaya fruits by *Mycosphaerella* transformants into which *Fusarium* cutinase gene was introduced. (1) Wild type *Mycosphaerella* spp.; (2) transformant M-101; (3-5) transformants M-12, M-25 and M-21, respectively; (6) transformant M-101 with rabbit antibodies prepared against *Fusarium* cutinase. The picture was taken 72 h after keeping the intact papaya fruits with the spore suspension (5×10^4 spores in 50 μl) on it at 26°C in a 100% humid chamber as before[5].

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1. Maiti, I. & Kolattukudy, P. E. *Science* **205,** 507-508 (1979).
2. Kolattukudy, P. E. *A. Rev. Phytopath.* **23,** 223-250 (1985).
3. Soliday, C. L., Flurkey, W. H., Okita, T. W. & Kolattukudy, P. E. *Proc. natn. Acad. Sci. U.S.A.* **81,** 3939-3943 (1986).
4. Soliday, C. L., Dickman, M. B. & Kolattukudy, P. E. *J. Bact.* **171,** 1942-1951 (1989).
5. Dickman, M. B., Patil, S. S. & Kolattukudy, P. E. *Physiol. Plant Pathol.* **20,** 333-347 (1982).
6. Kolattukudy, P. E. *Science* **208,** 990-1000 (1980).
7. Woloshuk, C. P. & Kolattukudy, P. E. *Proc. natn. Acad. Sci. U.S.A.* **83,** 1704-1708 (1986).
8. Dhawale, S. S., Paietta, J. V. & Marzluf, G. A. *Curr. Genet.* **8,** 70-79 (1984).
9. Kaster, R. K., Burgett, S. G. & Ingolia, T. D. *Curr. Genet.* **8,** 353-358 (1984).
10. Turgeon, B. G., Garber, R. C. & Yoder, O. C. *Molec. cell. Biol.* **7,** 3297-3305 (1987).
11. Lin, T. S. & Kolattukudy, P. E. *J. Bact.* **133,** 942-951 (1978).
12. Podila, G. K., Dickman, M. B. & Kolattukudy, P. E. *Science* **242,** 922-925 (1988).
13. Kolattukudy, P. E., Podila, G. K. & Mohan, R. *Genome* **31,** 342-349 (1989).
14. Walton, T. J. & Kolattukudy, P. E. *Biochemistry* **11,** 1885-1897 (1972).
15. Ptashne, M. *Sci. Am.* **260,** 40-47 (1989).
16. Cathala, G. *et al. DNA* **2,** 329-335 (1983).

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Molecular basis of lipid transfer protein deficiency in a family with increased high-density lipoproteins

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PLASMA high density lipoproteins (HDL) are a negative risk factor for atherosclerosis. Increased HDL is sometimes clustered in families, but a genetic basis has never been clearly documented[1]. The plasma cholesteryl ester transfer protein (CETP) catalyses the transfer of cholesteryl ester from HDL to other lipoproteins and therefore might influence HDL levels[2]. Using monoclonal antibodies, we show that CETP is absent in two Japanese siblings who have markedly increased and enlarged HDL. Furthermore,

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They are homozygous for a point mutation in the 5′-splice donor site of intron 14 of the gene for CETP, a change that is incompatible with normal splicing of pre-messenger RNA[3]. The results indicate that the family has an inherited deficiency of CETP due to a gene splicing defect, and illustrate the key role that CETP has in human HDL metabolism.

A Japanese subject has recently been described with markedly increased HDL and low levels of cholesteryl ester transfer activity[4]. Low activity could reflect a deficiency of CETP, the presence of an inhibitor[5], or a substrate effect (high HDL)[6]. Our goals were to assess blood and plasma samples from the Japanese subject and his family for the presence of the CETP of relative molecular mass (M_r) 74,000 (74K)[7], using two different CETP monoclonal antibodies[8,9], and also to analyse the defect at the DNA level using the sequence of the wild-type CETP gene (L.B.A. *et al.*, manuscript in preparation).

CETP in plasma was immunoaffinity-purified using an anti-CETP monoclonal IgG (TP2) column[8] and the retained fraction was analysed by western immunoblotting of SDS-polyacrylamide gels (Fig. 1). In the control subjects, in T.Y. (proband's spouse) and in the four children (Y.Y., K.C., M.D., K.Y.), CETP ran at its characteristic position[7]. Remarkably, CETP was not detected in the plasma of the proband (S.Y.) or of his sister (K.H.) (Fig. 1), even when 300 μl of S.Y. plasma was analysed (S.Y.×3) (Fig. 1). Also, when equal amounts of control and S.Y. plasma were mixed, there was no diminution of the CETP signal following immunoaffinity chromatography (Fig. 1; con+S.Y.).

To quantitate CETP more precisely, plasma samples were analysed by radioimmunoassay using ^{125}I-labelled TP2. In this competitive displacement assay[10], the plasma of S.Y. and K.H. did not displace the standard CETP from the solid phase, which is consistent with no CETP being present (Table 1). The amount of plasma CETP from the children ranged from 38 to 56% (mean 49%) of their mother's value (Table 1), which is consistent with their being obligate heterozygotes for inherited CETP deficiency, with codominant expression determining CETP levels.

In a further attempt to detect CETP in the S.Y. plasma, it was electrophoresed in native polyacrylamide gradient gels and either stained with Sudan Black to show the lipoproteins (Fig. 2a) or transferred onto nitrocellulose and probed with a monoclonal antibody that recognizes a different epitope, ^{125}I-labelled TP6 (Fig. 2*b*). In the lipid-stained preparations, normal HDL appears as a set of diffusely stained bands corresponding to particles of diameter between 8 to 12 nm (Fig. 2*a*; controls 1-4), and low-density lipoproteins (LDL) run as an intense band near the top of the gel. The proband (S.Y.) shows markedly increased and enlarged HDL, extending into the size region of LDL (Fig. 2*a*). Similar results were obtained with his sister (K.H.) (data not shown). Two of the children (K.C. and Y.Y.) showed a distinct increase in staining of the larger HDL subclass, and all showed an increase in HDL-2 (10-12 nm) relative to the smaller HDL-3 (8-10 nm). Thus, the HDL_2/HDL_3 ratio was above normal in the children (Table 1).

Western immunoblot analysis of plasma run on native polyacrylamide gradient gels and probed directly with ^{125}I-labelled TP6, showed that the CETP was present in a diffuse band co-migrating with HDL-3, with a similar distribution in control samples and in samples from T.Y. and the four children (Fig. 2*b*). But, no CETP could be detected in the plasma of either

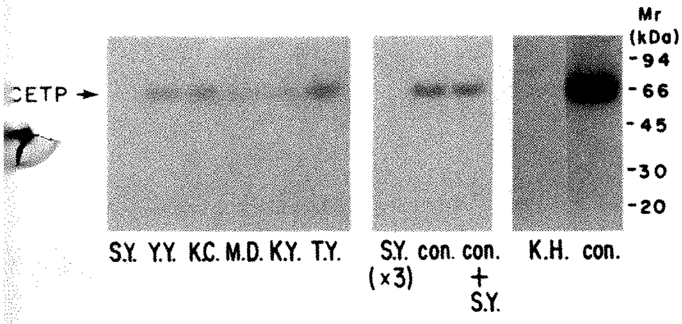

FIG. 1 Western immunoblot analysis of the anti-CETP immunoaffinity-retained fraction of plasma. Results are shown for the proband (S.Y.), his spouse (T.Y.), their four children (Y.Y., K.C., M.D., K.Y.), normolipidemic control subjects (con) and also for S.Y.'s sister (K.H.). The 'S.Y. (×3)' incubation contained 300 μl S.Y. plasma and the 'con+S.Y.' incubation consisted of 100 μl each of control and S.Y. plasma; all other incubations contained 100 μl plasma from the subject indicated. Autoradiographs (3-day exposure, except for K.H. and control, which is a 6-day exposure) of these blots are shown. The position of the 74K CETP is indicated as well as the positions of marker proteins (Pharmacia).

METHODS. Plasma samples containing 1 mM PMSF were subjected to immunoaffinity chromatography on an anti-CETP monoclonal IgG(TP2) column as previously described[8]. Recovery of CETP in the retained fraction under these conditions was ~80%. For western immunoblots, the retained fractions were subjected to SDS-PAGE, transferred to nitrocellulose and incubated with ^{125}I-labelled (Iodobeads-Pierce) anti-CETP monoclonal antibody TP2 (5×10^6 c.p.m./per ml).

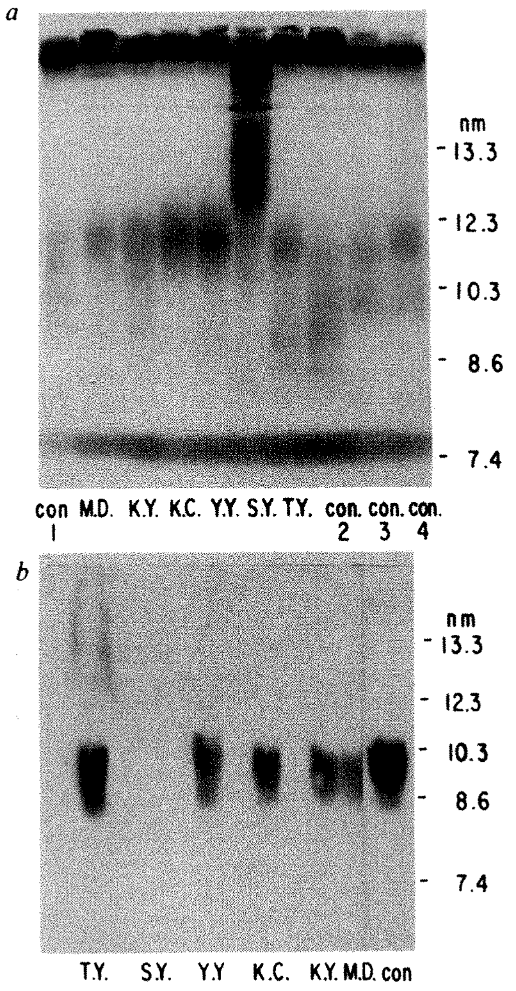

FIG. 2 Native, gradient polyacrylamide gel *a*, stained for lipid with Sudan Black, or *b*, western immunoblotted using anti-CETP monoclonal antibody TP6. *a*, The lipoprotein profile of the S.Y. family (see legend to Fig. 1) and 4 different normolipidemic controls was visualized by Sudan Black staining of a 4-30% gradient acrylamide gel (Pharmacia). Each lane represents 30 μl plasma electrophoresed for 18 h in Tris-borate buffer, pH 8. *b*, Western immunoblot analysis of 30 μl plasma from S.Y. and other family members after reaction with ^{125}I-labelled anti-CETP monoclonal antibody TP6. The hydrodynamic diameters (in nm) were estimated from the mobility of Pharmacia protein standards, which are shown on the right.

TABLE 1 HDL and cholesteryl ester transfer protein in the SY family

		Age/Sex	HDL-chol (mg dl^{-1})	APOA-I* (mg dl^{-1})	HDL2/HDL3†	CETP Activity (% control)	CETP‡ Mass (μg ml^{-1})
Proband	S.Y.	64 M	248	239	—	0	0
Siblings	K.H.	60 F	175	147	—	0	0
	T.A.	57 F	55	129	—	35	—
	Y.K.	47 M	63	—	—	105	—
Spouse	T.Y.	58 F	64	—	1.3	110 ± 10	2.5 ± 0.6
Children	M.D.	35 F	52	98	2.3	60 ± 12	1.0 ± 0.2
	K.C.	33 F	65	132	4.1	62 ± 5	1.3 ± 0.3
	Y.Y.	31 F	77	132	5.2	80 ± 14	1.4 ± 0.3
	K.Y.	28 M	52	98	4.1	45 ± 16	1.2 ± 0.4
Normolipemic (mean ± s.d.) Controls			48 ± 13	121 ± 22	0.99 ± 0.20		2.0 ± 0.5
	range		35-65	83-155	0.7-1.7		1.2-2.7
	N		19	19	15		12

* Determined by radial immunodiffusion.
† Determined by scanning Sudan-stained native gels (mean of duplicate determination).
‡ Determined by radioimmunoassay (mean ± s.d. of 3 separate assays).

S.Y. (Fig. 2*b*) or his sister (data not shown). Thus, analysis of plasma with two different antibodies against CETP (TP2 and TP6) (ref. 9) indicated an absence of CETP in both S.Y. and K.H.

This unusual lipoprotein phenotype raised the question of whether CETP deficiency was a secondary phenomenon or the result of an inherited defect of the gene for CETP. Preliminary Southern blot analysis using the CETP complementary DNA[11], revealed no major insertions or deletions of the CETP gene in the proband (results not shown). Therefore, the proband's genomic DNA (S.Y.) was used as a substrate for amplification of the 16 exons and exon/intron junctions of the CETP gene by the polymerase chain reaction (PCR)[12], in which we used intronic oligonucleotide primers positioned 50 to 100 nucleotides from the junctions. The amplified products were subcloned and sequenced[13]. The sequenced exons and exon/intron junctions encompassing the entire CETP gene (including the polyadenylation region and the translation initiation codon) were conserved when compared with the wild-type sequences (L.B.A. *et al.*, manuscript in preparation), with one exception. At the 5′ splice donor of intron 14 (position + 1) there was a G → A change altering the strictly conserved G-T intron splice donor[14] to A-T. This change was identified in three different subclones derived from separate PCR amplifications of the proband's DNA.

The G → A mutation was confirmed and familial inheritance documented by direct sequencing of amplified DNA from all available family members (Fig. 3). The amplified exon 13,14 PCR product was directly sequenced using a primer originating within exon 14. Figure 3 shows the DNA sequencing ladder in the region of the exon 14/intron 14 junction. Columns 1 and 2 represent the homozygous CETP-deficient individuals (K.H. and S.Y.) and the arrow indicates the A at the first position (+1) of intron 14. Columns 3, 4, 5 and 6 represent the children of the proband and they are heterozygotes with both an A and a G at this position of the sequencing ladder. The mother (T.Y.) of the heterozygotes is represented under column 7 with the normal G at this site. The first nucleotide of a normal intron is never an A (ref. 15). Mutations of the strictly conserved G at this position prevent normal splicing[16]. The position +1 G is critical for correct splicing as it is involved in binding U1 small nuclear ribonucleoprotein particles (U1 snRNPs) and is the residue that undergoes cleavage in the spliceosome and lariat formation[17]. A similar G → A mutation in the conserved splice donor site in intron 1 or intron 2 results in two forms of β-thalassemia. Both forms have abnormal β-globin mRNA and no β-globin protein[18,19]. In some cases, splice site mutations lead to activation of nearby cryptic splice sites[20] or use of an alternative normal splice site (exon skipping)[21], resulting in a truncated transcript and/or abnormal protein. The epitope of TP2 is at the C-terminus of CETP[9], corresponding to a region of the CETP gene downstream of the mutation. But CETP is also undetectable with the TP6 antibody (Fig. 2*b*), whose epitope maps between amino acids 261 to 367 of CETP[9]. This region of CETP is encoded by exons proximal to the site of the mutation. Thus, even if an alternative splice site is used in the mutant CETP gene, no CETP polypeptide is found in plasma. Th dinucleotide ^{m}CpG is under-represented[22] in the genome, but contributes to most (>35%) mutations characterized by single-base changes[23]. We speculate that the CETP deficiency of S.Y. and K.H. might have involved a hot-spot deamination on the non-coding strand.

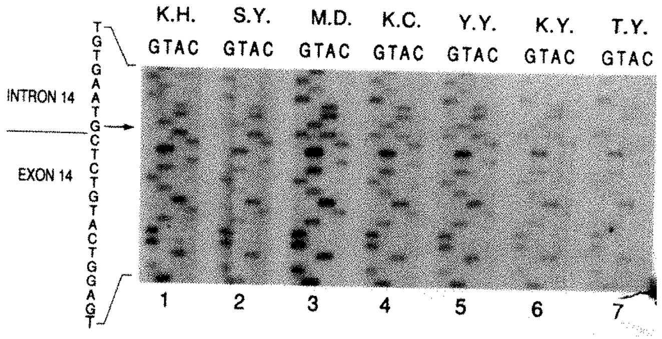

FIG. 3 DNA sequence ladder generated from the amplified products at the exon 14/intron 14 junction of the CETP gene in a family with CETP deficiency. Columns 1 and 2 (K.H. and S.Y.) of the autoradiograph (96 h exposure) represent the sequence of the homozygous CETP-deficient individuals. The arrow indicates the A residue observed in both K.H. and S.Y. Columns 3, 4, 5 and 6 (M.D., K.C., Y.Y. and K.Y.) show the heterozygotes with both an A and a G at position +1 of the intron. Column 7 (T.Y.) shows the sequence of the mother's DNA.

METHODS. Genomic DNA was isolated[24] from 10 ml blood and used as a substrate for amplification by PCR[12]. Polymerase chain reactions were carried out in a total volume of 100 μl under mineral oil, and contained 3 μg genomic DNA, 100 pmol primer A (5′-CCTGAGCTATGAGACAAAAG-3′, located 92 nucleotides 5′ of exon 13 of the gene for CETP), 100 pmol primer B (5′-AAAAGGTGAAATGGGAAGCT-3′ located 43 nucleotides 3′ of exon 14 of the gene for CETP), 2.5 U *Taq* polymerase (Cetus), 200 μM each dNTP, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% gelatin. The amplified products (30 cycles at 94°C for 0.5 min, 55°C for 1 min, 74°C for 3 min) were purified by Centricon-30 (Amicon) filtration and sequenced with a ^{32}P-labelled exon 14 primer (5′-CCAGAGCTTCCTGCAGTCAA-3′ located 56 nucleotides 5′ of the exon 14/intron 14 boundary) according to the Higuchi modifications of the 'Sequenase' USB protocol[25]. The sequencing reactions were heat-denatured and resolved on a 6% polyacrylamide gel containing 7 M urea[26].

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THE JOB The appointee will undertake research on chloroplast development The project involves the molecular cloning of two nuclear genes from maize that regulate chloroplast development Both genes are being tagged with transposable elements to facilitate cloning Once cloned, the role of each gene product in leaf development will be determined This project will be part of a larger research program studying environmental and developmental regulation of nuclear genes that code for chloroplast proteins

THE PERSON Applicants should possess a PhD or equivalent qualifications, research achievement in molecular biology, genetics, biochemistry or a related field and experience with DNA cloning are essential Experience with eukaryotic genetics and a knowledge of plant development and cell biology would be an advantage

CONDITIONS Appointment will be for a term of 18 months with possibility of an additional 24 months Australian Government Superannuation benefits are available

MORE INFORMATION Prospective applicants are invited to telephone Dr W C Taylor on 6162 46 5223 for further information A copy of the detailed duty statement and selection criteria may be obtained by telephoning 6162 46 5206

APPLICATIONS Applications should be submitted by 5 January 1990 and quote reference number N8925 They should be framed against the selection criteria, and state relevant personal particulars including details of qualifications and experience Applicants should give details of at least two referees, and address their applications to

The Chief
CSIRO
Division of Plant Industry
GPO Box 1600
CANBERRA ACT 2601
AUSTRALIA

(W6741)A

CSIRO
AUSTRALIA

AB1 PLN8925

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

POSTDOCTORAL TRAINING IN CELL REGULATION

The Department of Biochemistry, St Jude Children's Research Hospital, announces a training program in the molecular and cellular biology of cell growth, differentiation and transformation The program emphasizes a multidisciplinary approach that develops skills in biochemistry, molecular genetics and cell biology Fellows benefit from training in energetic, well-funded laboratories within an expanding research institute We offer competitive stipends plus the benefit of living in a growing, economical, sunbelt community Specific research groups participating in the program include

W Y Cheung	Regulatory properties and biological functions of calmodulin, Ca^{2+} regulated enzymes
J Cleveland	Growth factor signal transduction and regulation of gene transcription
V A Fried	The ubiquitin system and post-translational regulatory pathways
J N Ihle	Mechanisms of hematopoietic stem cell growth regulation, differentiation and transformation
S Jackowski	Regulation of phospholipid metabolism and the production of lipid-derived second messengers
C Rock	Cell cycle regulation of membrane biogenesis
E Thomas	Antimicrobial and antitumor mechanisms of leukocytes

Further information on individual research programs can be obtained by writing the Department of Biochemistry Applicants should indicate the research group(s) they are interested in, submit a brief statement of their research interests, curriculum vitae, reprints, and names of three references to

Postdoctoral Training Program
Department of Biochemistry
St. Jude Children's Research Hospital
332 N Lauderdale
Memphis, TN 38105

An Equal Opportunity/Affirmative Action Employer

(NW4568)A

UNIVERSITY OF CALIFORNIA, DAVIS
BODEGA MARINE LABORATORY

POPULATION BIOLOGIST

The Bodega Marine Laboratory, a research unit of the University of California, Davis, invites applications for a position in population biology The position is open at the tenured level — associate and full professor Applicants must have a Ph D (or equivalent), and demonstrated excellence in their fields We particularly encourage scientists to apply whose research will take advantage of the diverse ecosystems of the Northern California coast where the Laboratory and its surrounding natural reserve are located In addition to research, teaching and advising responsibilities, the appointee will serve as the Laboratory's coordinator for research and instruction in population biology, alongside counterparts from organismal biology and aquaculture and fisheries The position is at the Laboratory full time, although the appointee will also be a member of an appropriate department on the Davis campus

Applicants should provide a letter of research and teaching interests, *curriculum vitae*, two selected reprints, and the names, addresses and phone numbers of three references to **Dr James B Clegg, Bodega Marine Laboratory, P O Box 247, Bodega Bay, CA 94923**

The Selection Committee will begin review of applications on 15 January 1990, the position will remain open until filled

The University of California is an Equal Opportunity/ Affirmative Action Employer (NW4631)A

A leading pharmaceutical Company actively involved in new drug discovery in a range of therapeutic areas, is seeking a

HEAD OF DRUG METABOLISM

He or she will lead a group dealing with the disposition and metabolism of candidate compounds in animals and man

This group is in the Department of Pharmacokinetics and Metabolism, of R & D division, based in Italy

Applicants should have a PhD in chemistry, biochemistry or in a biological area, with several years post-doctoral experience in drug metabolism and a sound knowledge of the role of drug metabolism studies in drug development

Candidates should send a curriculum vitae including a list of publications, names and addresses of references with **Rif A–1639 evidenced on the envelope** to

Sintex

SINTEX S r l — 20146 MILANO —
Via Frua 22 —ITALY (W6738)A

COLD SPRING HARBOR LABORATORY

FACULTY POSITION
X-RAY CRYSTALLOGRAPHY

We seek applicants for a newly-created faculty position in the field of protein structure and function, particularly X-ray crystallography, which will expand an ongoing effort at Cold Spring Harbor Current faculty members in this area include Dr. Jim Pflugrath, Dr John Anderson and Dr Jeff Kuret The X-ray crystallography group will move into the new W M Keck Foundation Structural Biology Laboratory in 1991 Both senior and junior faculty positions will be considered

Curriculum vitae and names of references should be submitted to

Dr. Bruce Stillman
Cold Spring Harbor Laboratory
P.O. Box 100
Cold Spring Harbor, NY 11724

CSH **Cold Spring Harbor Laboratory**

An equal opportunity employer

(NW4606)A

CITY University

DEPARTMENT OF OPTOMETRY AND VISUAL SCIENCE

Manager-Electron Microscopy
To £23,826

This is a new and exciting post responsible for running and developing the commercial potential of our electron microscopic laboratory This houses a JEOL JEM 1B instrument with facilities for transmission, scanning and X-ray analysis Your responsibilities will include maintaining equipment, liaising with users, service work, and instructing staff and project students without user experience

As well as managing University-related activities, you will have the flair to market commercial opportunities with other academic and industrial concerns—mainly in the biological tissue area

Experience in the use of EMs and back-up equipment is essential, and knowledge of biological structure would be a distinct advantage Salary will be on the scale £20,340-£23,826 inclusive of London Allowance A performance related bonus scheme is under active consideration This appointment is for 3 years in the first instance

For further details, please contact Professor G L Ruskell, Department of Optometry and Visual Science, City University, Northampton Square, London EC1V 0HB Tel 01-253 4399, Ext 4305

(2160)A

Free University of Brussels —
Institute of Interdisciplinary Research
Profs J.E Dumont – G Vassart

POST DOCTORAL POSITION
TRANSGENIC MICE PROGRAM

A two year position, starting immediately, is available to participate in the development of a newly set up transgenic mice team in Brussels The research project will involve the following themes

- Oncogenesis of the thyroid
- Oncogenic potential of specific viral genes
- Development of homologous recombination in ES cells, with the aim of generating transgenic models of receptor deficiency

Applicants should have a strong background in molecular biology techniques Additional experience in microinjection techniques, cell culture, or ES cells handling would be an advantage

Applications, including a C V and names of two referees, should be sent to **Dr M Parmentier, I R I B H N., ULB Campus Erasme, 808 route de Lennik, 1070 Brussels, Belgium** (02/526 41 72) (W6739)A

Institute of Terrestrial Ecology

MOLECULAR BIOLOGIST/ PLANT POPULATION ECOLOGIST

Furzebrook, Dorset

A population geneticist with interest and experience in the application of DNA technology to ecological and population studies is required The successful candidate will join a small team at ITE Furzebrook working on the population ecology and genetics of a range of eukaryotes and will be expected to apply DNA techniques to natural populations of plants, (initially *Brassica oleracea*) to investigate population substructuring and gene flow He/she will also be required to assist in the technical training of staff working on relatedness in animal populations, and the development of molecular methods within the Institute

Applicants will be expected to have been awarded a PhD in a plant genetic subject Experience with RFLP analysis and DNA fingerprinting and the development of DNA probes is vital

The appointment is for three years Salary will be in the range of **£10,678** to **£14,909** per annum, or **£13,254** to **£18,864** per annum depending upon qualifications and experience Benefits include generous holidays and a non contributory pension scheme

The Institute of Terrestrial Ecology is a component body of the Natural Environment Research Council

Further information and application forms may be obtained from the Station Secretary, Institute of Terrestrial Ecology, Furzebrook Research Station, Wareham, Dorset BH20 5AS, or by telephoning 09295-51518/9

Closing date for receipt of application forms, including a full curriculum vitae, is 7th December 1989

The Natural Environment Research Council is an Equal Opportunities Employer

Natural
Environment
Research
Council

(2175)A

ASSOCIATE BIOLOGIST

Transgenic mouse expert — experienced in generating transgenic mice and in manipulating mouse embryos Duties will include overseeing operation of transgenic facility, teaching postdoctoral and doctoral investigators techniques involved in injecting, maintaining and screening transgenic animals and adapting and developing new approaches to genetic transfer experiments in mice Applicant will also aide in day-to-day operation of facility and the execution of critical experiments Salary will be commensurate with qualifications and experience Please send resume to **Dr Carol Readhead, California Institute of Technology, Division of Biology 147-75, Pasadena, Ca, 91125**
EOE/AA (NW4601)A

UNIVERSITAT FUR BODENKULTUR WIEN

Am Zentrum fur Angewandte Genetik der Universitat fur Bodenkultur, Wien, ist die neugeschaffene Planstelle einer/eines

Ordentlichen Universitätsprofessorin/ Universitatsprofessors für Angewandte Genetik

ab sofort zu besetzen.

Der **Aufgabenbereich** umfasst den Aufbau facherubergreifender, grundlegender Lehrveranstaltungen auf dem Gebiet der Allgemeinen Genetik, insbesondere der Molekuar- und Zeligenetik Daruber hinaus ist die methodisch-experimentelle Ausbildung der Studenten aller Studienrichtungen besonders zu berucksichtigen Die Forschungstatigkeit der Bewerberin/des Bewerbers soll auf experimentelle Systeme in hoheren Eukaryonten ausgerichtet sein Enge Kooperation mit anderen Instituten der Universitat im Rahmen angewandter Forschungsprojekte wird erwartet

Ernennungserfordernisse sind eine der Verwendung entsprechende inlandische oder gleichwertige auslandische Hochschulbildung sowie eine an einer osterreichischen Universitat erworbene oder gleichwertige auslandische Lehrebefugnis oder eine der Lehrbefugnis als Universitatsdozent gleichzuhaltende wisenschaftliche Befahigung fur das Fach, das der zu besetzenden Planstelle entapricht

Bewerbungen sind unter Beifugung eines Lebenslaufs, einer Darstellung des wissenschaftlichen Werdeganges sowie der bisherigen und beabsichtigten Arbeitsgebiete, ferner einer Liste der wissenschaftlichen und sonstigen Veroffentlichungen (mit sonderdrucken besonders signifikanter Arbeiten) sowie einer Darstellung der bisherigen Lehrtatigkeit bis spatestens *31 Janner 1990*

an den Rektor der Universitat fur Bodenkultur, Gregor-Mendelstrasse 33, A-1180 Wien, zu richten (W6745)A

UNIVERSITY OF NOTTINGHAM

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH ASSISTANT

A post-doctoral research assistant is required to join a group studying early embryonic development The project is to investigate the biological role of homeobox genes in development using molecular biology and surgical manipulation of chick embryos

The position is for 3 years, funded by the Wellcome Trust and is available from January 1990

Applications including the names and addresses of two referees, should be sent as soon as possible to the **Secretary, Department of Biochemistry, University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH** For informal enquiries telephone Dr P Scotting on 0602-709367 (2133)A

UNIVERSITY OF EDINBURGH
FACULTY OF MEDICINE

LECTURESHIP (NON-CLINICAL) IN SURGERY

ROYAL INFIRMARY OF EDINBURGH

Applications are invited for a Non-Clinical Lecutreship in the Department of Surgery at the Royal Infirmary of Edinburgh Candidates should have a BSc (Hons) degree and a PhD The successful candidate will be required to participate in a departmental research programme investigating mediators of altered protein metabolism in disease states Experience in tissue culture techniques and immunoassay would be advantageous

Salary will be at an appropriate point on the Non-clinical University Lecturers scale AT2A (£10,458-£15,372) or scale AT2B (£16,014-£20,469), according to age, qualifications and experience USS Superannuation

Further particulars may be obtained from the **Personnel Office, University of Edinburgh, 63 South Bridge, Edinburgh EH1 1LS** with whom applications (12 copies), including curriculum vitae and the names and addresses of two referees should be lodged not later than December 31st 1989

Please quote reference number 1482 (2144)A

UNIVERSITY OF
essex

Department of Biology

LECTURESHIP IN CELL BIOLOGY OR IMMUNOLOGY

pplications are invited for the post of Lecturer in the ›epartment of Biology (salary on Grade A, £10,458 to 15,372 per annum or Grade B, £16,014 to £20,469 per nnum) for appointment as soon as possible for a period of ıree years

Ve are seeking a scientist with complementary interests in ell biology to join a very active research department, well quipped for a wide range of modern immunological, ellular and molecular techniques

nformal enquiries about this post may be made to ›rofessor J G M Shire, tel (0206) 873307

\pplications (ten copies), including curriculum vitae ınd the names and addresses of two referees, should each the Registrar (L/990/N), University of Essex, Nivenhoe Park, Colchester, Essex, CO4 3SQ by 15th)ecember 1989 Further particulars of this post may ›e obtained by telephoning Colchester (0206) 872462 24 hours) (2132)A

THE LONDON HOSPITAL MEDICAL COLLEGE
(University of London)

DEPARTMENT OF IMMUNOLOGY

POSTS IN MOLECULAR IMMUNOLOGY

A research post funded by the Multiple Sclerosis Society for up to 3 years The project involves sequencing of HLA genes and the analysis of T cell receptors The work will complement cellular and functional investigations and the generation of T cell clones already under way in MS and other associated disorders There would be opportunity to participate in these related areas For further details contact Dr J Sachs on 01 377 7750 or Dr A Dos Santos on 01 377 Ext 3320

A research post funded by the Cancer Research Campaign for up to 3 years The project involves the study of the biological significance of HLA Class I-like molecules and their relation to human tumours A variety of experimental procedures will be used including biochemical, molecular biology and cellular techniques For further details contact Dr Biro on 01 377 7000 Ext 3322

lary on the University 1A scale according to age and experience Letter of ›lication together with CV and the names of two referees should be sent to e **Secretary, The London Hospital Medical College, Turner Street, London E1 D** Applicants could be considered for both posts but may express their ference Both posts are available immediately (2137)A

MEDICAL RESEARCH COUNCIL
COLLABORATIVE CENTRE

POSTDOCTORAL SCIENTIST MOLECULAR VIROLOGIST

postdoctoral scientist is required to join an MRC AIDS Directed ogramme sponsored project participating in the development of fective chemotherapeutic drugs against HIV infection The successl applicant will be responsible for studies of the mechanism of ːtion of selected agents and related investigations of particular ages of HIV infection, as well as the development of assays for hibitors of defined targets Experience with recombinant DNA techques is desirable

ıe position will be tenable for 3 years The salary will be in the range 12,381 to £20,469 (starting salary dependent on experience) plus 1,650 per annum London Allowance MRC Pension Scheme option

pplicants including a curriculum vitae, list of publications and the ames and addresses of two professional referees should be sent to **Ir C R Russell, Administrative Manager, National Institute for ledical Research, The Ridgeway, Mill Hill, London, NW7 1AA,** not ıter than Friday 15th December quoting reference number CC/ADP/ D

he MRC is an Equal Opportunities Employer (2155)A

RHM
RHM RESEARCH AND ENGINEERING LTD

HEAD OF BIOCHEMISTRY

Do you have a good honours degree in Biochemistry or Chemistry followed by a PhD and at least five years postdoctoral experience? Can you demonstrate a good track record in the application of your science? Do you have experience of managing other scientists? Right now we may have the opportunity for which you have been waiting which will allow you to combine your scientific and personal skills to meet the challenges and opportunities of the 90's in the food manufacturing industry

Ranks Hovis McDougall is a major international manufacturer of foods as Hovis, Mr Kipling, Shredded Wheat, Robertsons, Bisto, Sharwood, and the new mycoprotein Quorn

The Lord Rank Research Centre, pleasantly situated in the Chiltern Hills at High Wycombe, is the corporate multidisciplinary research and development organisation for RHM and provides a lively, stimulating and well equipped working environment

We wish to recruit a Senior Biochemist to lead our team of scientists undertaking studies of the chemistry and biochemistry of raw materials and products

Reporting to the Head of Food Science you will be expected to initiate, develop and apply fresh ideas to our project studies as well as managing and developing the skills and career aspirations of the people in the Biochemistry Section You must therefore have a creative mind, good communication skills and the ability to win the respect of colleagues through leadership and scientific excellence

The RHM Group offers its scientists the rewards, training and career development opportunities expected of a major international company

Interested? The send your CV, quoting reference 513, to

Personnel Department,
RHM Research and Engineering Limited,
The Lord Rank Research Centre,
Lincoln Road,
High Wycombe,
Bucks HP12 3QR

Tel (0494) 26191 (2174)A

Search Reopened
University of Nebraska-Lincoln
Computer Science and Engineering Department
Department Chair

Seek applicants and nominees to chair dynamic department that has 15 doctorate faculty and offers programs leading to the BS, MS, and Ph D degrees Research programs are in algorithms, theoretical computer science, communication and information theory and networks, information retrieval, fault tolerant computing, and human factors UNL has a wide variety of computing resources linked by a sophisticated campus-wide network It is the lead institution in the NSF-funded regional network, MIDnet, and a node on the NSFnet backbone Special state tax funds have been appropriated to enhance research

Requires Doctorate in Computer Science or related field and evidence of strong leadership for research and academic programs Administrative experience is desirable

Qualified applicants should send resumes and names of three references postmarked by February 1 (or until suitable candidates apply thereafter) to **Samuel B Treves, Associate Dean, College of Arts and Sciences, 1223 Oldfather Hall, University of Nebraska-Lincoln, Lincoln, Nebraska 68588-0312**

Affirmative Action/Equal Opportunity Employer (NW4599)A

POST-DOCTORAL SCIENTIST TRANSCRIPTIONAL CONTROL MECHANISMS

Amgen, a leader in the development of innovative human therapeutics through applied biotechnology, has a post-doctoral position available in the laboratory of Dr Grant A Bitter to study control of eukaryotic gene expression The interaction of defined mammalian DNA regulatory regions with their cognate protein factors will be investigated When appropriate, *Saccharomyces cerevisiae* will be used as a surrogate host

Qualified applicants must have a Ph D in a relevant discipline and be experienced in recombinant DNA methodology, protein purification and methodologies for studying protein-nucleic acid interactions Experience in the genetics of *S cerevisiae* is a plus

Amgen offers an unique opportunity to participate in challenging research programs using state-of-the-art technology in an environment that fosters scientific creativity and collaboration If you have the necessary skills and qualifications and would like to experience research in a supportive company environment, please send a C V, in confidence, to

AMGEN, INC
Recruitment, Dept #432
Amgen Center
Thousand Oaks
CA 91320-1789

(NW4621)A

Equal Opportunity Employer

AMGEN

RESEARCH ASSOCIATE

Duties include independent research o
central nerve system (CNS) extracellul
matrix and its influence on spinal co
injury Also responsible for directin
the protein chemistry laboratory an
technicians engaged in the isolation
brain extracellular proteins, the chara
terization of their sugar content, the
association with the cell membranes a
well as their degradation after spin
cord injury and their role in CNS regen
eration Lastly, responsible for th
preparation of cell cultures in order t
create a c-DNA library for cloning an
molecular biology studies

Special requirements include a Ph D i
Biochemistry and two years of postdoc
toral studies with experience as demon
strated by scientific publications in th
isolation of proteins from CNS extra
cellular matrix, protein-membran
interactions, protein-liposome interac
tions and circular dichroism Als
required is experience in immunohisto
chemistry, electron microscopy, cel
culture of glia cells and HPLC

Position located in Boston, Massachusetts Salary £25,000 per annum, 40 hours per week Send resume only to

Job Order #00074
Massachusetts Department of
Employment and Training
C F Hurley Building
Boston, Massachusetts 02114

(NW4623)A

UNIVERSITY OF OXFORD

10 UNIVERSITY LECTURESHIPS IN CHEMISTRY

Applications are invited for ten lectureships in all branches of chemistry including its borders with the biological and physical sciences Two of these posts are to be held in the Inorganic Chemistry Laboratory (associated with Fellowships at Balliol and St Hilda's or Trinity Colleges), five in the Organic Chemistry Laboratory (with Fellowships at Brasenose, Exeter, Hertford, Lady Margaret Hall and St Peter's Colleges), and three in the Physical Chemistry Laboratory (with Fellowships at Magdalen, St Catherine's and St Hilda's or Merton Colleges)

Intending applicants should write for further particulars as soon as possible to the Head of *one* of the laboratories **Professor M.L.H Green, Inorganic Chemistry Laboratory, South Parks Road, Oxford OX1 3QR, FAX (0) 865 272690; Professor J.E. Raldwin, Dyson Perrins Laboratory, South Parks Road, Oxford OX1 3QY, FAX (0) 865 275674, or Professor J.S. Rowlinson, Physical Chemistry Laboratory, South Parks Road, Oxford OX1 3QZ, FAX (0) 865 275410.**

Those interested in posts in more than one laboratory should mention this in their letter of enquiry It is not necessary, to write to the colleges The closing date for applications will be 14 January 1990 (2145)A

The University is an Equal Opportunity Employer

RESEARCH FELLOW/ SENIOR RESEARCH FELLOW

Part-time A$31,525-A$45,699

Division of Entomology

THE JOB External funds are being provided for the appointee to undertake revisionary work on the higher level taxonomy of the moth family Pyralidae, with special emphasis on the Phycitinae

THE PERSON Applicants should hold a PhD degree or equivalent qualification in a relevant field and have research experience in Lepidoptera taxonomy Some knowledge of other economically important groups of Microlepidoptera would be highly desirable

CONDITIONS Appointment will be for a term of three years and the appointee will be required to work 18 hours per week, at an hourly rate based on the above salaries

FURTHER INFORMATION Prospective applicants are invited to telephone Dr Ebbe Nielsen on 6162 46 5493 for further information A copy of the detailed job description and selection criteria can be obtained by telephoning Ms Jody Scrivener on 6162 46 5075 Facsimile enquiries can be directed to 6162 47 0217

APPLICATIONS Should be submitted by the 15 December 1989 and should quote reference number C0384 Applications should relate to the selection criteria and should provide details of relevant personal particulars, including qualifications and experience Applicants should nominate at least two professional referees and address their applications to

The Chief
CSIRO
Division of Entomology
GPO Box 1700
CANBERRA ACT 2601
AUSTRALIA

(W6740)A

CSIRO
AUSTRALIA

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

AB1 89/34

THE UNIVERSITY OF MELBOURNE AUSTRALIA

POST-DOCTORAL FELLOW

Steroid regulation of gene expression

osition available for studies
the steroid regulatory ele-
ients for the urokinase plas-
inogen activator gene A
rong background in mole-
ılar biology or protein chem-
try is desirable Send CV and
ıe names of three references
Dr J A Hamilton, Univer-
ty of Melbourne, Depart-
ent of Medicine, Royal
elbourne Hospital, Parkville,
ustralia, 3050 (W6737)A

POSTDOCTORAL POSITION

ne Genetics division of The Chil-
en's Hospital, Harvard Medical
chool, is seeking an individual to
ork on the role of genetic imprint-
ıg in the etiology of Angelman
nd Prader-Willi syndromes The
andidate should have a back-
round in molecular genetics and
keen interest in the application of
combinant DNA techniques to
ıe study of human genetic
isease
lease reply to **Dr Marc Lalande,**
ienetics Division, The Children's
ospital, Harvard Medical School,
00 Longwood Avenue, Boston,
1A 02115
qual Opportunity Employer
(NW4615)A

Institute of Terrestrial Ecology

COMMUNITY ECOLOGIST

Dorset

Applications are invited for the position of post-doctoral fellow to study the effects of fertilisers and liming on populations of grassland invertebrate animals, especially Hemiptera Particular attention will be given to interactions of fertilisers and liming with grassland management The work will include investigation of the ecology of selected species and intensive sampling of populations on experimental sites

The successful candidate will be based at Furzebrook Research Centre, but initially considerable use may be made of the Classical Field Experiments at Rothamsted Experimental Station, Harpenden Applicants will be expected to have been awarded, or be due to obtain, a Phd in an appropriate discipline and will have a good background in entomology, agricultural zoology or zoology Knowledge of sampling methodology and the design and analysis of field experiments would be an advantage

The appointment is for three years and salary will be in the range of **£10,678** to **£14,909** per annum depending on experience and qualifications The post offers generous holidays and a non contributory pension scheme A current driving licence is essential

The Institute of Terrestrial Ecology is a component body of the Natural Environment Research Council

Further information and application form may be obtained from the Station Secretary Institute of Terrestrial Ecology, Furzebrook Research Station, Wareham, Dorset, BH20 5AS, or by telephoning 09295 51518 Closing date for completed applications, including a full curriculum vitae is 14th December 1989

The Natural Environment Research Council is an Equal Opportunities Employer

Natural
Environment
Research
Council

(2179)A

MOLECULAR BIOLOGIST

ASSISTANT/ASSOCIATE PROFESSOR

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

Full-time faculty position/Department of Radiation Oncology, Radiation Oncology Research Laboratory (RORL) Working with RORL investigators, the molecular biologist will develop research to complement and extend existing projects in cell biology, cell kinetics, biochemistry, and radiation biology Our program is multi-disciplinary in nature, emphasizing areas relating to radiation, hyperthermia and chemotherapy The position includes teaching duties, but no clinical responsibility Salary is commensurate with education and experience Send cv to **Gloria C Li, RORL, CED-200, UCSF, San Francisco, CA 94148-0808** *UCSF is an EOE/AA employer Women and minorities encouraged to apply* (NW4630)A

BIOLOGY:

The Department of Biology of the Pennsylvania State University invites applications from candidates with a Ph D who have research interests in evolutionary ecology, molecular evolution, and/or comparative physiology Tenure track positions are available at any level, depending upon the candidate's qualifications, positions will be available beginning August, 1990 or January, 1991 Applicants should have demonstrated research ability, postdoctoral experience, and interest in undergraduate and graduate education We are especially interested in persons who will interact productively with members of our existing group of ecologists, molecular evolutionists, population biologists and physiologists

Applications/nominations along with curriculum vitae, statement of research interests and teaching goals, and the names and addresses of three references should be addressed to **Dr Linda Maxon, Head, Department of Biology, Box C, 208 Mueller Laboratory, University Park, PA 16802** Applications/nominations will be accepted until December 10, 1989, or until the position is filled *An Affirmative Action/Equal Opportunity Employer Woman and Minorities Encouraged to Apply* (NW4618)A

POSITION NOTICE

HEAD

Department of Veterinary Pathobiology, College of Veterinary Medicine, University of Illinois at Urbana-Champaign

Position available February 1, 1990, or until filled

Qualifications DVM or equivalent degree, and/or PhD in a biological area The applicant or nominee must have had organizational and administrative experience

Duties The incumbent will be responsible for the execution of departmental, College, and University policies and shall provide direction for departmental activities (research, professional and graduate teaching, and services)

Applications The search process to fill this position has been extended In order to ensure full consideration, applications must be received by December 15, 1989 Applications, including three letters of reference should be submitted to

Dr Gary Jackson,
Chairman, Search Advisory Committee,
Office of the Dean,
College of Veterinary Medicine,
University of Illinois,
2001 South Lincoln Ave ,
Urbana, Illinois 61801
Telephone (217) 333–2760

The University of Illinois is an Affirmative Action/Equal Opportunity Employer (NW4610)A

CELL BIOLOGY

DEVELOPMENTAL NEUROBIOLOGY

Two senior level faculty positions

Department of Biology
University of California, Riverside

The Department of Biology, University of California, Riverside, invites applications for two openings at the senior level. One is in cell biology, at the Associate or Full Professor level; the other is in developmental neurobiology at the Full Professor level The appointments will be effective July 1, 1990. Applicants should have an active research program, and an established reputation with a proven record of competing successfully for extramural funding. Demonstrated interest and ability in teaching is sought Salary will be competitive and will depend on qualifications and experience. Modern research space and access to major equipment such as TEM, SEM, and staffed biotechnology and analytical chemistry instrumentation facilities are available.

CELL BIOLOGY

We are interested in all areas of research in cell biology. Preference will be given to scientists applying modern molecular approaches to the study of the cell and with interests in: cell/cell interactions, protein sorting and assembly, cytoskeletal structure and function, or extra-cellular matrix. Applications should be received by January 15, 1990. Please send *curriculum vitae,* summary of research and teaching interests, and arrange to have 3-5 referees send letters to:

Cell and Molecular Biology
Search Committee,
Department of Biology,
University of California,
Riverside, CA 92521.

(NW4632)A

CONDENSED MATTER EXPERIMENTAL PHYSICISTS

COLUMBIA UNIVERSITY
DEPARTMENT OF PHYSICS

The Department of Physics of Columbia University is searching for outstanding condensed matter experimental physicists at the assistant professor level

The successful candidates will be provided with startup funds to suit the requirements of their research programs There is no restriction on the subfield that will be considered Tenure positions will be available for some of the physicists in this program who develop truly outstanding research programs and who also demonstrate excellent teaching skills We will also consider applications, at this time, from more senior experimental condensed matter physicists who presently have the qualifications for a tenure position

Finally we will consider applications from small, established, highly successful groups of experimental physicists who might be interested in moving as a group to Columbia These positions will remain open until they are filled Persons or groups interested in being considered for these positions should send their curriculum vitae, publication list, and a statement of their research interests to

Professor S. Nagamiyn
Condensed Matter Search Committee Chairman
Department of Physics
Columbia University
Box 3 Pupin
New York, N.Y. 10027

They should also arrange to have, at least three letters of recommendation sent to the same address

Columbia University is an equal opportunity-affirmative action employer Women and members of minority groups are encouraged to apply for these positions (NW4624)A

NATIONAL FOOD BIOTECHNOLOGY CENTRE
UNIVERSITY COLLEGE, CORK

The National Food Biotechnology Centre (NFBC) was established in 1987 as a partnership between University College Cork and BioResearch Ireland (a division of EOLAS)

RESEARCH SCIENTISTS and RESEARCH ASSISTANT

The Centre now has vacancies for Research Scientists to work on a number of projects, which are under development in the following areas

1 The development of genetic marker systems for the detection of specific Gram-negative microorganisms

1 Senior Scientist (Molecular Genetics)

2 Biochemical, physiological and genetic characterization of lactic acid bacteria involved in dairy fermentations

1 Senior Scientist (Microbiology/Microbial Biochemistry)
1 Research Assistant (Microbiology/Biochemistry)

3 Characterization of the genes involved in biological control in *Pseudomonas* species

1 Senior Scientist (Molecular Biology/Molecular Genetics)

Applicants for the Research Scientist positions should hold or expect to hold a PhD degree or have equivalent experience in the appropriate disciplines outlined above Applicants for the Research Assistant post should hold a recognised qualification in Microbiology or Biochemistry

These positions are supported by funding from EC research and development programmes, multinational industries and EOLAS (The Irish Science and Technology Agency) Posts will initially be offered as one-year renewable contracts and salary will be competitive and commensurate with experience Successful applicants should be prepared to start work as soon as possible Applicants should submit a C V and arrange that two letters of reference be sent not later than Friday, 15 December, 1989, to

Professor F O'Gara, Director, National Food Biotechnology Centre, University College Cork, Ireland
Tel (353-21) 273803 (2170)A

Coláiste na hOllscoile Corcaigh
University College Cork

CULTY POSITION IN HARMACEUTICAL SCIENCES

NIVERSITY OF WASHINGTON, SEATTLE, WA., U.S A.

Department of Pharmaceutics invites appli-
ns for a tenure-track position at the Assistant
essor level As part of an ongoing expansion
ır department, we are seeking an individual
strong research training in biopharmaceu-
cell physiology, biochemistry, or biophysics,
is interested in developing a basic research
ıram relevant to problems of drug delivery
disposition, in particular for protein or
tide-based drugs The preferred areas of
arch include **biomembrane transport, endo-**
tic processes, protein/peptide or liposome
ractions with biomembranes, and intracellu-
istribution and metabolic mechanisms The
artment currently has well-funded and inter-
ve research programs in pharmacokinetics
drug metabolism Advanced bioanalytical
rumentation, including FAB-tandem mass
ctrometer and high-field NMR, is available
in the School of Pharmacy The position will
olve teaching at both the graduate and under-
duate levels We are particulary interested in
ority and women applicants

ters of application, including curriculum vitae,
atement of research interests, and three letters
reference should be sent to **Danny D Shen,**
D, Chairman, Search Committee, Department
armaceutics, BG-20, University of Washing-
, Seattle, WA 98195, U S A Application will
accepted until 15 February, 1990

e University of Washington is an equal oppor-
ity, affirmative action employer (NW4600)A

ASSISTANT/ ASSOCIATE PROFESSOR

nure tract position, to join the Tumor
ology group in the Department of Patho-
gy and Laboratory Medicine at the
niversity of Florida, Gainesville Indivi-
ual must have an M D and/or Ph D
legree and established research produc-
ivity in an area of tumor biology or tumor
mmunology Excellent research environ-
nent for fundamental studies, unique
pportunities for close collaboration with
aculties of the Pathology, Surgery and
Medical Oncology services, and an on-
going cancer biology training program for
doctoral candidates, and post-doctoral
ellows Salary is competitive and related
o qualifications and experience Applica-
ion deadline is December 31, 1989 with an
anticipated start date of January 31, 1990

Send CV, bibliography, and two recent
eprints of your work in referred journals
to

Richard T Smith, M D
University of Florida
Department of Pathology
and Laboratory Medicine
Box J 275, JHMHC
Gainesville, Florida, 32610

Affirmative action/equal opportunity employer (NW4603)A

SCIENTIFIC DIRECTOR SUPERCOMPUTER FACILITY

The National Cancer Institute's supercomputer facility is a totally integrated Class VI computer center devoted to problems in biomedical research and is managed by Program Resources, Inc at the Frederick Cancer Research Facility in Frederick, Maryland Users have convenient access to a CRAY X/MP 24 supercomputer, VAX minicomputers (8600 and 11/785), SUN, Apollo, Silicon Graphics, Evans and Sutherland graphics workstations, and a variety of microcomputers all networked by an Ethernet local area network High speed communications networks extend user access to other research organizations and universities through the nationwide internets (ARPANET, NSFNET, BITNET) Research applications include Sequence Analysis, Structure Prediction, Molecular Graphics, Image Processing, Structure Determination, Crystallography, MRI, Statistical Analysis, and Data Base Analysis

The candidate we seek will be responsible for directing and coordinating the scientific programs of the supercomputer facility which has a broad user base focusing on contemporary problems in biomedical research with special focus on the NCI's mission in Cancer and AIDS research

This facilty is being considered for upgrade, thus the Scientific Director will have a major role in the establishment of design and instrumentation upgrades and will have an opportunity to carry out research consistent with broad program goals The Scientific Director will be supported by an outstanding incumbent technical and administrative staff of 29 employees

Ph D or M D degree with demonstrated expertise in drug design, 8-10 years of related experience and a national reputation directing scientific programs is required along with detailed knowledge and direct experience with supercomputer hardware and technology

PRI offers a salary commensurate with credentials and a comprehensive company benefits program including a 401(k) Retirement Plan and relocation assistance

For consideration please send resume, references, publications list, and salary requirements on behalf of the Supercomputer Scientific Director Search Committee to

Robert E Hardisty
Personnel Manager
PROGRAM RESOURCES, INC
NCI-FCRF
P O Box B
Frederick, MD 21701

(NW4614)A

PRI PROGRAM RESOURCES, INC

Equal Opportunity Employer, M/F

AFRC INSTITUTE FOR ANIMAL HEALTH
PIRBRIGHT, WOKING, SURREY GU24 0NF

MOLECULAR BIOLOGIST/ VIROLOGIST (SENIOR SCIENTIFIC OFFICER)

to work on

MOLECULAR BIOLOGY AND IMMUNOLOGY OF RINDERPEST VIRUS — A 5 YEAR RESEARCH PROJECT FUNDED BY THE WELLCOME TRUST

Rinderpest virus is a serious veterinary pathogen, closely related to measles and canine distemper virus, which affects ruminants in many developing countries It is an excellent model for the study of virus pathogenicity in the natural host at the molecular level since strains differing greatly in their virulence have been isolated

A SENIOR SCIENTIFIC OFFICER is required for research on the analysis of the structure and function of virus proteins using molecular biological techniques

Minimum qualifications A good degree in an appropriate subject plus a PhD and 4 years relevant experience

Salary £13,254-£18,864 according to qualifications and experience Non-contributory superannuation scheme

Informal enquiries can be made by phone (0483 232441) or letter to Dr T Barrett or Dr C J Bostock

Applications with curriculum vitae and the names of two referees to the Laboratory Administrator at the above address (2131)A

MICROBIOLOGIST

ASSISTANT, ASSOCIATE, OR PROFESSOR

The Department of Microbiology, University of California at Davis invites applications for an 11-month, tenure-track, open rank position from candidates who have demonstrated excellence in microbiological research The candidate will join a strong, supportive and growing Department with strength in diversity, physiology and genetics Teaching responsibilities include graduate and/or undergraduate courses in bacterial physiology and genetics Please send a curriculum vitae, selected publications, a statement of proposed research, a description of teaching experience and interests, and the names of three references to **Dr J C Meeks, Chair, Physiology/Genetics Search Committee, Department of Microbiology, University of California, Davis, CA 95616**

The position will remain open until filled, however, to be assured of consideration, applications should be received by February 1, 1990

The University of California is an Affirmative Action/Equal Opportunity Employer Women and Minorities are encouraged to apply (NW4597)A

UNIVERSITY OF EDINBURGH

Department of Microbiology

POST-DOCTORAL RESEARCH FELLOW

Applications are invited for an MRC funded post-doctoral research assistant position in the laboratory of Dr M P Gallagher The project involves molecular characterisation of the nucleoside permeases of *Salmonella typhimurium*, using current techniques of molecular genetics/biology, immunology and biochemistry The system serves as a model for cancer therapy and it is hoped that the study will unravel key features of permease assembly and substrate specificity, as well as regulation of permease synthesis in this organism Experience in bacterial genetics, molecular biology (cloning and sequencing) or membrane biochemistry is preferable but not essential

The position is for three years and is available immediately but starting date is negotiable for an appropriate candidate Starting salary will be between £10,458 and £12,381 depending on age and experience

Informal enquiries and applications, including C V and the names and addresses of two referees, to **Dr M P Gallagher, Department of Microbiology, University of Edinburgh, Kings Buildings, West Mains Rd , Edinburgh EH9 3JG, U K** (telephone 031-6671041 xt 268 or FAX 031 667 2601) by 15 December, 1989

PLEASE QUOTE REFERENCE NO 5726 (2158)A

UNIVERSITY OF DURHAM

Department of Geological Sciences

SENIOR RESEARCH ASSISTANT IN SPACE GEODESY

Applicants are invited for the post of Post Doctoral Research Assistant in Global Positioning System (GPS) Geodesy, to participate in the research effort at Durham, and be responsible for the processing of GPS data Candidates should hold a Ph D degree and preferably have a background in Geodesy and Computing Consideration will also be given to applicants with a background in Geophysics Informal enquiries concerning the appointment may be made to Dr G R Foulger (tel 091–374–2514)

The appointment, which is funded by the Wolfson Foundation, will be from early 1990 for up to three years The salary will be within the Research and Analogous 1A scale (£10,458–£16,665), according to experience

Further particulars may be obtained from the Registrar, Science Laboratories, South Road, Durham, DH1 3LE, tel 091–374–2265, to whom applications (three copies, including full CV and the names of three referees) should be sent not later than Friday, 29th December, 1989 Please quote ref GL3 (2126)A

THE UNIVERSITY OF LEEDS

Department of Earth Sciences

RESEARCH ASSISTANT/FELLOW

Applications are invited for the above post for a fixed period of two years for work on geophysical research, especially in geomagnetism applied to the Earth's deep interior and the geophysical fluid dynamics of the Earth's core, involving the use of a SUN4 computing system, as part of a first rate research group

A good honours degree in Mathematics, Physics or Geophysics is required, and further qualifications/experience in computing, particularly FORTRAN programming and the use of UNIX-based systems, would be an advantage

Salary for Predoctoral Research Assistant on R/A Grade IB (£9,816–£12,381) or, for Postdoctoral Research Fellow, on R/A Grade IA (£10,458–£16,665) according to qualifications and relevant experience Candidates for Research Fellow should have a PhD or equivalent experience

Informal enquiries may be made to Dr K A Whaler (tel (0532) 335216)

Application forms and further particulars may be obtained from, and completed applications forwarded to, **The Registrar, The University, Leeds LS2 9JT** (tel (0532) 333969 — direct line), quoting reference no 49/41

Closing date for applications 14 December 1989 (2128)A

UNIVERSITY OF CANTERBURY

New Zealand

SENIOR LECTURER O LECTURER IN MOLECULAR BIOLO

(Department of Plant anc Microbial Sciences)

Applications are invited for a ition, as above Applicants sh have a Ph D degree in a rele field and an interest in plant m cular biology and plant virol The appointee will be expecte contribute to the teaching courses in Biochemistry, Molec Biology, Molecular Genetics Virology and to pursue an ac research programme including supervision of M Sc and P students

The salary for Senior Lectu is on a scale from NZ$50,00 NZ$58,600 per annum (bar), a range from NZ$60,800 NZ$64,500, and for Lecturer on a scale from NZ$36,000 NZ$43,700 (bar), and fr NZ$45,000 to NZ$47,200 annum

Further particulars and C ditions of Appoinment, quo Position No PL5, may be obta from Appointments (372 Association of Commonwea Universities, 36 Gordon Squa London WC1H 0PF

Applications close with Registrar, University of Cant bury, Private Bag, Christchur New Zealand, on 28 Febru 1990

The University has a policy equality of opportunity in empl ment (W6734)A

UNIVERSITY OF SOUTHAMPTON

LYMPHOMA RESEARCH UNIT

Postdoctoral Position

Post-doctoral scientist or equiva lent, required to join a projec concerned with recognition o idiotypic immunoglobulin on lymphoma cells by cloned hos anti-idiotypic T cells This projec forms part of a large investiga tion of therapeutic approache to B-cells lymphoma usin natural effector mechanisms

Applicants should have previou experience in the general area of immunology or biochemistry Salary will be on the RA1A scale up to £12,879 per annum

Informal enquiries may be made to Dr F Stevenson, tel no 0703 702636, to whom applications should be sent at the Lymphoma Research Unit, Tenovus Laboratory, Southampton General Hospital, Southampton, SO9 4XY, by 14 December 1989 **Please quote reference number 148/JAD/jm**

(2169)A

Faculty Position in
VIROLOGY

.e Department of Microbiology, North iroltna State University, invites applints for a tenure-track position as Assistit Professor in Virology A Ph D and levant postdoctoral experience in plant, icterial or animal virology are required ie successful candidate is expected to ›velop a vigorous research program in a isic aspect of virology and participate in 'aduate and undergraduate teaching The ›sition is a 12-month tenure-track appointent that includes appropriate startup nds and continuing institutional research ıpport Excellent opportunities exist for ›llaboration with other virologists on ımpus and in the Research Triangle Area he application should include a statement f research interest, curriculum vitae with list of publications and the names, ddresses and telephone numbers of three əferences Application materials should e sent to

Chairman
Virology Search Committee
Department of Microbiology
Box 7615
North Carolina State University
Raleigh, NC 27695-7615

he closing date for applications is January [illegible], 1990 Proper documentation of identity and employability will be required before he hiring process can be finalized North Carolina State University is an equal pportunity employer (NW4612)A

ELECTRON MICROPROBE ANALYST
and
TRANSMISSION ELECTRON MICROSCOPIST

The University of Alabama is seeking an electron microprobe analyst to operate a new JEOL 8600 electron microprobe (EMP) and a transmission electron microscopist to operate a new Hitachi H-8000 transmission electron microscope (TEM) The EMP is equipped with 5 wavelength dispersive spectrometers, TN 5600 automation, a TN 5402 energy dispersive X-ray analyzer, and a TN 5702 digital image analysis system The TEM is equipped with a STEM attachment, a TN 5502 X-ray detector, and a signal processing system Both instruments are located in the central analytical facility of the new Tom Bevill Energy, Mineral, and Material Science Research Building which houses a variety of electron optical, X-ray, spectrographic, and chromatographic analytical equipment

The EMP analyst and TEM microscopist will supervise operation of the instruments, perform analyses for researchers, and train users in routine analytical procedures Experience in the following areas is considered an asset for the EMP analyst 1) microprobe techniques and X-ray data correction methods for geological materials, 2) mineral chemistry and petrology, 3) computer programming, and 4) EMP maintenance Experience in the following areas is considered an asset for the TEM microscopist 1) TEM/STEM techniques for materials science, 2) thin-film research, 3) quantitative analytical microscopy, and 4) high-resolution microscopy The EMP analyst and TEM microscopist are expected to participate in on-going research projects and will be encouraged to carry-out their own research

The EMP analyst should have a M S in Geology, but candidates with expertise in some or all of the above fields will be considered The TEM microscopist should have a degree in Natural Sciences or Metallurgy, work experience in a research environment is desirable Salaries will be commensurate with experience Applications must be received before February 1, 1990, appointments begin March 1, 1990 Send a letter of application, vitae, and the names of three references to **Dr Jen-Ho Fang, Chairman, Department of Geology, Box 870338, University of Alabama, Tuscaloosa, Alabama 35487**

The University of Alabama is an equal opportunity/affirmative action employer

(NW4611)A

FULL PROFESSOR
VISION RESEARCH

Applications and nominations are invited for a tenured faculty research position at the level of Professor in the Eye Research Institute of Oakland University The candidate must be an established investigator with Ph D , M D or equivalent, a minimum 10 years experience in independent research, a strong publication record and, where appropriate, proven ability to secure continuous external grant support We are particularly interested in established scientists in molecular biology or immunology who have, or will make, a strong commitment to vision research Investigators in other areas of biomedical sciences who will make the same commitment will also be considered

Opportunities exist for collaboration with current faculty who are engaged in research in the biochemistry, physiology and molecular biology of the cornea, lens, retina and intraocular fluids In addition, collaboration on clinical research projects is also possible with faculty and residents of the affiliated Department of Ophthalmology of a major area hospital

The Eye Research Institute is an independent academic unit of Oakland University, a state supported institution situated on a 1400 acre campus, 25 miles north of Detroit, with an enrollment of 9500 students in baccalaureate and doctoral programs

Applicants should send a curriculum vitae, 3 to 5 reprints and the names of three references by February 16, 1990 to **Dr M V Riley, Chair, Search Committee, Eye Research Institute, Oakland University, Rochester, MI 48309**

An Equal Opportunity/Affirmative Action Employer (NW4617)A

BROWN UNIVERSITY
ASSISTANT PROFESSOR OF MEDICAL SCIENCE (ARTIFICIAL ORGANS AND BIOMATERIALS)

For a full-time regular position as Assistant Professor of Medical Sciences we are seeking a broadly trained chemical engineer/polymer scientist whose research interests are in the area of biomaterials with emphasis on surface modification and tissue-material interaction A Ph D or equivalent degree is required Some biology background and postdoctoral research experience are preferred The position is linked to the Artificial Organ/Biomaterials Program, a multidisciplinary group of faculty and graduate students, with current emphasis on tissue-material interactions, bioresorbable polymers, and the development of membrane-protected tissue transplants Candidates should demonstrate outstanding potential to develop an independent externally funded research program The position requires teaching survey courses in the field of polymer science and biomaterials and guidance of Ph D and M D -Ph D students The appointment can begin between July 1 and December 31, 1990

To ensure full consideration, applications should be received by February 16, 1990 Applicants should submit curriculum vitae, a set of representative reprints, a concise description of research interests and goals, and should arrange for three letters of reference to be sent to **Professor E H Leduc, Division of Biology and Medicine, Box G, Brown University, Providence, RI 02912** *We encourage applications from women and minority candidates Brown University is an Equal Opportunity Affirmative Action Employer* (NW4636)A

Department of Neurobiology
Duke University Medical Center

Applications are invited for 11 new tenure track positions at all ranks in the newly created Department of Neurobiology at Duke University Medical Center investigators will occupy research space in the Joseph and Kathleen Bryan Neurobiology Building scheduled for completion in February 1990 and will be leaders in university wide graduate and undergraduate programs in neuroscience Applicants with strong research records are being solicited from the full range of sub fields in neurobiology including molecular cellular systems, and behavioral approaches Minimal qualifications include an M D or Ph D degree and several years of research experience in neurobiology The positions will be available from the fall of 1990

Applicants should submit a curriculum vitae a statement of research interests and objectives and letters from at least 3 references to Dr Dale Purves, Chairman, Department of Neurobiology, Box 3209, Duke University Medical Center, Durham, North Carolina 27710

Duke University offers highly competitive salaries and benefits Eligible employees enjoy outstanding educational opportunities including educational assistance and tuition grants for children (NW4609)A

Duke University

A Universe Of Opportunity

Duke University is an Equal Opportunity/Affirmative Action Employer

Celgene

MICROBIOLOGIST/BIOCHEMIST CHEMIST/ENZYMOLOGIST

Celgene Corporation is a leader in the application of biotechnology to the chemical industry The recent development of novel proprietary technology has led to the creation of two high-profile, post-doctoral research opportunities within its **CHIRAL PRODUCTS PROGRAM.**

These positions provide opportunities to gain industrial research experience in the application of biocatalysis to the resolution and synthesis of commercially-valuable chiral molecules The successful applicants will be highly motivated individuals who will develop new areas of research as part of a multidisciplinary group These positions will appeal particularly to scientists with research experience or interest in microbial product formation or the chemistry of enzyme action

Celgene Corporation is a well-funded public company committed to becoming the premier name in chemical biotechnology Qualified candidates should send a detailed resume, indicating position of interest to **Personnel Department NM, Celgene Corporation, 7 Powder Horn Drive, Warren, NJ 07060.**

An Equal Opportunity Employer M/F (NW6435)A

UNITED MEDICAL AND DENTAL SCHOOLS OF GUYS AND ST THOMAS"S HOSPITALS

VIROLOGY **RESEARCH ASSISTANT**

required to join a team at St Thomas's Campus and the University of Surrey to study the immunodominant epitopes of rubella virus and to produce recombinant and synthetic antigens Experience of virological techniques essential, and molecular biological techniques and advantage PhD desirable, but not essential Further information from 01 928 9292 ex 2453 Salary in range £9,816–£12,381 per annum plus £1,650 London Weighting To apply send CV and names of two referees to the **Personnel Officer, UMDS, St Thomas's Campus, Lambeth Palace Road, London SE1 7EH** quoting ref STH/VIR/451 by 14th December 1989 (2161)A

UNIVERSITY OF BRISTOL
DEPARTMENT OF PHYSIOLOGY
LECTURESHIP

Applications are invited for the above post on the Grade A scale (£10,458-£15,372) or Grade B scale (£16,014-£20,469), depending on age and qualifications, from active and successful researchers who are also enthusiastic teachers The department's current research interesrts are in four main areas membrane and cell biophysics, muscle and motility, sensory mechanisms in the CNS and motor control The department teaches Physiology and Histology to Science, Medical, Veterinary and Dental students, and would expect the new appointee to be willing to teach in any area

Shortlisted candidates will be expected to deliver to the department a first-year undergraduate lecture of their choice on January 18th and a short seminar on their research on the morning before interview on January 19th

Applications, in the form of a detailed curriculum vitae with an explanatory letter giving names and telephone numbers of three referees, should be sent to the **Registrar, Senate House, Bristol, BS8 1TH,** to be received by January 5th 1990 Please quote reference CYF 2

Further particulars from Dr J Luck, Physiology Department, Bristol University, BS8 1TD, telephone (0272) 303465, who will be happy to show potential applicants round the department

An equal opportunities employer (2125)A

ST. MARY'S HOSPITAL MEDICAL SCHOOL

(a constituent College of Imperial College of Science,
Technology and Medicine)
(University of London) Norfolk Place, LONDON W2 1PG

POSTDOCTORAL RESEARCH ASSISTAN

DEPARTMENT OF ANATOMY & CELL BIOLOGY

required for a project funded by the Wellcome Trust on the developmen mammalian motoneurones The work will involve labelling and morphome analysis of motoneurone dendrites during normal postnatal development a after disruption of neuromuscular interaction Experience with neuroanatomi tracing methods is desirable but not essential

The post is for 1 year in first instance renewable with a starting salary up £14,703 pa + London Allowance For further information telephone Dr M Lowrie (01-723 1252 Ext 5352 or 5342)

Applications in form of a full C V with names and addresses of 2 referees shou be sent to Personnel Department at above address by 15 December Plea quote Ref PRA/AN (2138)A

UNIVERSITY OF EDINBURGH
DEPARTMENT OF VETERINARY PATHOLOGY
LECTURESHIP IN PATHOLOGY

Applications are invited from graduates in Veterinary Medicine for the abo lectureship The successful candidate will be expected to participate fully research, teaching and diagnostic work of the Department The individu appointed will be joining a Department with multi-disciplinary interests in tl Paraclinical Sciences The person appointed will carry out a share of routi diagnostic pathology, be responsible for an area of teaching in veterinary pat ology at undergraduate and Honours level and will be expected to develop research programme in the paraclinical area

This post is funded under the New Academic Appointments Scheme with effe from early 1990 Salary will be on Lecturer A Scale (£10,458-£15,372)

Further particulars may be obtained from Professor I McConnell, Department Veterinary Pathology, University of Edinburgh, Summerhall, Edinburgh, EH 1QH (tel 031 667-1011, Ext 5285) with whom applications, including curriculu vitae and names of three academic referees, should be lodged no later than 18t December, 1989 (ref NAAS 2009) Overseas applicants should register intere by telex and send one copy of their application Interviews for the post will b held in early January (2143)A

POSTDOCTORAL POSITION
HARVARD UNIVERSITY

A postdoctoral position in theoretical atmospheric chemistry is availabl immediately The successful candidate will join a large research grou conducting theoretical and experimental investigations of a broad rang of issues in atmospheric chemistry He/she will participate in curren efforts to simulate global tropospheric chemistry using a general circulation model He/she will also have opportunities to become involved i other projects, including photochemical model studies and analyses o field data Independent research will be encouraged

A strong background in atmospheric science, chemical engineering, environmental engineering, or physical chemistry is required Previous experience in scientific computing is a plus

Interested persons should send a curriculum vitae and the names and addresses of three references by December 31, or until position is filled, to **Dr Daniel Jacob, Department of Earth and Planetary Sciences, 29 Oxford St , Harvard University, Cambridge, MA 02138**

Harvard University is an equal opportunity/affirmative action employer

(NW4575)A

ADVERTISEMENT:
Director/Assistant or Associate Scientist

The University of Kansas Biochemical Research Resource Unit seeks a Director for its Biochemical Research Service Lab The Director is responsible for the day-to-day operation of the lab which provides specialized research service to the University research community The Director will supervise a staff of two technicians Laboratory activities include protein purifications, enzyme kinetics, amino acid analysis, DNA sequencing, cloning and site directed mutagenesis Applicants should possess a Ph D in biochemistry, microbiology, chemistry, or a related discipline and post-doctoral experience in genetic engineering To apply send letter, resume and three letters of recommendation to **Charles J Decedue, Executive Director, Higuchi Biosciences Centers, 2095 Constant Avenue, University of Kansas, Lawrence, Kansas 66047 Consideration of applications will commence December 15, 1989, but applications will be accepted until July 30, 1990, or until the position is filled**

An Equal Opportunity/Affirmative Action Employer (NW4598)A

The NERC British Institutions Reflection Profiling Syndicate (BIRPS)
University of Cambridge, Department of Earth Sciences

Directorship of BIRPS

Applications are invited for the Directorship of BIRPS from April 1990, ollowing the retirement of the present Director, Dr D H Matthews, FRS The Director and Core Group of BIRPS are funded by the Natural Environment Research Council, and the current NERC grant will run or a *further 3 years to April 1993 The Director will provide scientific* eadership for the Core Group, including 4 post doctoral research workers, collaborate with syndicate scientists from other institutions, liaise with NERC and the industrial supporting partners in BIRPS The Director will be a member of the syndicate advisory committee defining *new directions for the Group in the next funding period* The Core Group is located at the Bullard Laboratories, University of Cambridge, providing close liaison with members of the Earth Sciences Department

Applications should be sent to the **BIRPS Directorship Search Committee, c/o Administrator, Department of Earth Sciences, Downing Street, Cambridge CB2 3EQ, U K** *by 15 January, 1990* (2154)A

THE MEDICAL COLLEGE OF ST. BARTHOLOMEW'S HOSPITAL DEPARTMENT OF GASTROENTEROLOGY

RESEARCH ASSISTANT

A Graduate Research Assistant with experience in Molecular Biology is required for a 2 year position to develop gene probes for use in the *diagnosis and molecular epidemiology of the intestinal protozoal* parasite of man, *Giardia lamblia* The candidate should have a degree in biochemistry, molecular biology or related subjects Salary is on the Research Assistant Scale 1B £9,816 £12,381 per annum) plus London Weighting of £1,650 Opportunity to register for a higher *degree may be possible Informal enquiries welcome Applications* including a full CV and names of 2 referees should be forwarded to **Dr Michael Farthing, Department of Gastroenterology, West Smithfield, London EC1A 7BE Tel No 01-601 8509**

Closing date 14 December 1989 (2139)A

University of Oxford Institute of Molecular Medicine

Post-doctoral Research Assistant

The above Post is available for 2 years from 1 January 1990 for an individual to join a team studying the Molecular Mechanisms controlling activation of Phospholipase A_2 and Phospholipase C Applicants should have a background in Biochemistry or Cell Biology and experience in Protein or Lipid Biochemistry *would be advantageous but is* not essential The salary will be on the 1A scale with the starting point being up to £13,527 Applicants should write, enclosing a full C V *and the names and addresses of two referees, to*

Dr C T Jones, Laboratory of Cellular and Developmental Physiology, Institute for Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DU

(2152)A

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nature

Still first with the best in science

University of Oxford
Physical Chemistry Laboratory

Surfactants and Biopolymers at Surfaces

Applications are invited for two postdoctoral research assistantships, one *funded by SERC and one by AFRC*

The SERC project is to use the *technique of specular neutron reflection to determine the struc*tures of polymers, surfactants, and surfactant mixtures at solid/liquid and liquid/air interfaces

The AFRC project is to apply the same technique to study biological molecules (phospholipids, proteins, etc) at interfaces Both projects will be based in Oxford but will involve experiments at Institut Lanc-Langevin, Grenoble, Rutherford Appleton *Laboratory and Brookhaven National Laboratory*

Candidates will be appointed within the 1A scale £10458 to £16665 and should send a curriculum vitae and names of two referees to **Dr R K Thomas, Physical Chemistry Laboratory, South Parks Road, Oxford OX1 3QZ, U K**

Applications are welcome from all EC countries or overseas

(2164)A

DEPARTMENT OF VISUAL SCIENCE
Institute of Ophthalmology Judd Street, London, WC1H 9QS

We have vacancies for

Postdoctoral Research Assistants

on work related to the transfer of the retinal input through subcortical visual areas

1 **ELECTROPHYSIOLOGIST** This position is for three years and is intended to study the mechanism(s) underlying the action of GABA in thalamic nuclei with special emphasis on the lateral geniculate and the perigeniculate nucleus using brain slice and single electrode voltage clamp techniques
2 **ELECTROPHYSIOLOGIST** This position *is for an initial period of 18 months and is intended to investigate the voltage activated* conductances underlying the rhythmic activity of thalamocortical cells activity using brain slice and single electrode voltage clamp techniques
3 **NEURONAL MODELLING** This position is for two years and involves the development of computer simulations of thalamocortical cells activity and *their modulation by retinal and cortical inputs*

   For positions 1 and 2 previous experience in *in vitro* electrophysiological techniques is advantageous but not essential For position 3 experience in computer modelling and knowledge of neuronal simulation techniques is highly desirable

These three positions are funded by The Wellcome Trust *with salary in the range* £14,703 £16,014 *plus* £1,650 London Allowance Applications, with curriculum vitae and the names of two referees, should be sent to Dr V Crunelli at the above address, from whom further particulars can be obtained (Tel 01 387 9621 ext 233)

4 **NEUROPHYSIOLOGIST** This position is *for three years and is funded by the MRC — project entitled "Transmitters of visual and somatosensory afferents* to the superior colliculus" Experience of neuro physiological and/or neuro pharmacological techniques (especially in relation to amino acid transmitters or sensory systems) would be an advantage, although not essential

For this position salary will be in the range £12,381 £13 527 plus £1 650 London Allowance Applications, *including curriculum vitae* should be sent to Dr T E Salt at the above address (Tel 01-387 9621 ext 231) (2162)A

THE SCHOOL OF PHARMACY UNIVERSITY OF LONDON

POST-DOCTORAL RESEARCH ASSISTANT

A Post-Doctoral Research Worker is required for a two year period

The successful applicant will have experience in phytochemistry and particularly in the isolation and structure determination of natural products The research to be carried out forms part of the collaborative programme established under the 1989 Award for the Advancement of European Science funded by the Korber Foundation It is proposed that the research will include whole plant material, plant tissue cultures and pharmacological investigations Enquiries to Professor J D Phillipson, Department of Pharmacognosy, The School of Pharmacy, 29-39 Brunswick Square, London, WC1N 1AX Tel 01 837 7651

Salary scale from £11,108 to £18,315 depending upon experience

Applications in the form of a CV and naming two referees should be submitted to the **Assistant Secretary** at the above address no later than 15 December 1989 (2136)A

MEDICAL RESEARCH COUNCIL NATIONAL INSTITUTE FOR MEDICAL RESEARCH

POSTDOCTORAL SCIENTIST

Applications are invited for a short-term (three year) postdoctoral position with Dr Jack Price in the Laboratory of Embryogenesis to work on cell lineage in the central nervous system using retroviral vectors

The work will involve molecular biology, tissue culture and neuroanatomy, some experience of one or more of these areas would be an advantage Further information about the nature of the work can be obtained from Dr Price on 01-959 3666 extension 2220 The salary will be in the range £12,381 to £20,469 (starting salary dependant on experience) plus £1,650 per annum London Allowance MRC Pension Scheme option

Applications including a curriculum vitae, list of publications and the names and addresses of two professional referees should be sent to **Mr C R Russell, Administrative Manager, National Institute for Medical Research, The Ridgeway, Mill Hill, London, NW7 1AA,** not later than 15th December 1989 *quoting reference number* PEG/0189

(2156)A

MOLECULAR GENETICIST

THE UNIVERSITY OF RICHMOND

invites applications for a tenure-track position in biology at the assistant professor level The primary teaching responsibility will be to coordinate and teach a core curriculum undergraduate course in molecular genetics and, on occasion, teach an undergraduate course in immunology In addition, the appointee will be expected to develop advanced undergraduate and MS-level courses in molecular genetics areas Preference will be given to those who will develop an active research program in molecular genetics involving undergraduate students Candidates must have the Ph D degree and post-doctoral experience is desirable

Submit resume, undergraduate and graduate transcripts, a statement of research plans and three letters of reference to **Dr F B Leftwich, Department of Biology, University of Richmond, VA 23173** Consideration of applications will begin on January 30, 1990

The University of Richmond is an equal opportunity/affirmative action employer and encourages applications from women and minority candidates (NW4605)A

EMORY UNIVERSITY

Faculty Positions in Developmental Biology

As part of a major expansion, the Department of Biology intends to make several tenure-track appointments in developmental biology Presently available positions are at the assistant professor level, although an appointment at a higher rank could be considered in exceptional circumstances The area of research interest is broadly defined to encompass the study of molecular mechanisms relevant to the fundamental processes of differentiation and morphogenesis Applicants should provide evidence that they will develop a strong, independent research program and that they possess a broad knowledge of developmental biology Applications from minority and female candidates are strongly encouraged

Please send a curriculum vitae, reprints of publications, a statement of present and future research plans, and four letters of recommendation to **Developmental Biology Search Committee, Department of Biology, Emory University, Atlanta, GA, 30322** To ensure full consideration, applications should be received by December 31, 1989

Emory is an Equal Opportunity/Affirmative Action Employer

(NW4607)A

The University of California, San Diego is seeking a Ph D applicant for a position of **Assistant Research Biochemist** Candidates should be a molecular genetist with experience in retrovirology, cell biology, histochemistry and neuroanatomy Focus of the research will be in the area of gene therapy in the central nervous system Salary range based on University of California policy Interested individuals should send curriculum vitae with a list of three references no later than December 15, 1989 **Theodore Friedmann, M D , Professor, Department of Pediatrics, UCSD/M034 La Jolla, CA 92093** *Equal Opportunity/Affirmative Action Employer* (NW4627)A

LABORATORY SPECIALIST

needed for studies involving viruses and viral DNA, virus isolation and infection, restriction enzyme analysis, Southern and Northern blotting, and molecular cloning

Salary \$7.80 per hour

Require Masters Degree in Molecular Biology, knowledge of molecular cloning techniques and viral biology and ability to use restriction analysis, gel electrophoresis and DNA hybridization Job Order No. 3941890

Contact **Utah Job Service, 1234 South Main Street, P.O. Box 11750, Salt Lake City, Utah 84147** (NW4602)A

UNIVERSITY OF HEIDELBERG

Centre for Molecular Biology Heidelberg (ZMBH

POSTDOCTORAL POSITION

Human molecular tumour genetics

Applications are invited for a two year postdoctoral positi
to participate in the ongoing molecular analysis of chrom
some 11p13 The focus of the work will be the identification
genes at or near the translocation breakpoint (11,14) (p13,1
in childhood T-ALL The successful candidate should have
strong background in cell biology, immunology and prote
chemistry Salary will be according to german BAT regulation

Please send full CV and the names of two referees to **Dr Royer-Pokora, Institut fur Humangenetik, Im Neuenheim Feld 328, 6900 Heidelberg, FRG,** by December 31, 1989

(W6747)A

MOLECULAR ENDOCRINOLOGY OF HAIR GROWTH

University of Glasgow

Dermatology Department

An immediate vacancy exists for a creative postdoctoral biochemist, molecular biologist in a new research group studying mechanisms of steroid hormone action in cells cultured from human hair follicles Appointment on scale 1A will be initially for one year, renewable for up to three years Starting salary up to £13,527 per annum

Application to **Dr M B Hodgins, Dermatology Department, Glasgow University, Anderson College Building, 56 Dumbarton Road, Glasgow, G11 6NU** (2130)A

UNIVERSITY OF HONG KONG

CLINICAL PATHOLOGIST

(Re-advertisement)

Applications are invited for the post of Clinical Pathologist in the Immunology Section of the Hospital Pathology Services, Department of Pathology Applicants should be medically qualified and post-registration experience in clinical medicine would be an advantage

Annual salary (superannuable) is on a 13-point scale HK\$219,120–446,040 (approx £17,810–36,260, sterling equivalent as at November 2, 1989) Starting salary will depend on qualifications and experience At current rates, salaries tax will not exceed 15% of gross income Children's education allowances, leave, and medical benefits are provided, housing or tenancy allowances are also provided in most cases at a charge of 7½% of salary

Further particulars and application forms may be obtained from Appointments (37199), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF, UK, or from the Appointments Unit, Registry, University of Hong Kong, Hong Kong, (Fax (852) 5-85852549) Closes 13 January 1990

(W6735)A

Postdoctoral position o
for

PROTEIN CRYSTALLOGRAPHI in BERLIN

for 2 years Can be extende
Prof W Saenger, Institut
Kristallographie, der Freien U
versitat Berlin, Takustr 6, D-1
Berlin 33 Telefax +30-838 67
(W6749)

AFRC INSTITUTE FOI ANIMAL HEALTH

SENIOR IMMUNOLOGI

As part of a strengthenin
immunological research the I
tute for Animal Health wishe
appoint a senior immunol
to its Division of Immunolog
Pathology The post-holder wi
based at the Compton Laborat

This new post will be concei
with developing a research
gramme on bovine T-cell rece
variable region genes and par
pation in an ongoing project on
avian histocompatibility comp
There will be ample opportu
for collaborating with others w
the Institute

Applicants should posses
higher degree in an immunolog
subject and have several years'
perience of research Experie
of farm animals is essential
working knowledge of molec
biology is desirable

The post is graded UG7/S
with a salary on the scale £17,3
£22,606 (UG7), £13,254-£18,
(SSO) There is a non-contribut
pension scheme The annual le
allowance is 25 days with an ad
tional 10 5 days for public holida
Assistance with removal expen
to a maximum of £5,000 may
available

Application forms and furtl
details are available from Mrs
Hughes, Personnel Officer, AF
Institute for Animal Heal
Compton, Berkshire RG16 0N
Tel (0635) 578411

The closing date for applicati
is 15/12/89 (2173)A

ASSISTANT PROFESSOR

pplications are invited for a position at the level of assistant
rofessor (tenure track) in the Department of Chemistry at Stan-
ord University, with primary research and teaching interests in
he general area of inorganic chemistry Applicants must be
rongly motivated toward creative research and have a com-
itment to teaching at the undergraduate and graduate levels In
pplying, please send curriculum vitae, list of publications, a brief
tatement of research interests (three pages or less) and, in
ddition, have three letters of recommendation sent to **Professor**
arden M McConnell, Chairman, Department of Chemistry,
tanford University, Stanford, California 94305

tanford is committed to Equal Opportunity through Affirmative
ction in employment, and we are especially eager to identify
inority persons and women with appropriate qualifications
pplications should be received by January 15, 1990 (NW4629)A

Postdoctoral Positions

ailable immediately for (1) syn-
etic organic chemist to study
olecular biological processing
oxidative DNA damages (2)
otein biochemist to study
echanism of action of DNA
pair enzymes (3) molecular
ologist to study regulation of
IA repair enzymes Campus-
ide, highly interactive DNA
pair group, enthusiastic, active
boratory, great environment

d CV and names of three
ferences to **Dr Susan Wallace,**
niversity of Vermont, Depart-
ent of Microbiology and Mol-
ular Genetics, Given Building,
urlington, Vermont, 05404
(NW4620)A

UNIVERSITY OF ST ANDREWS

Department of Biochemistry and Microbiology

POSTDOCTORAL RESEARCH ASSISTANT

pplications are invited for a
RC-funded postdoctoral posi-
n of up to 3 years to work on the
olecular genetics of nitrite reduc-
on in barley Applicants should
ave a background in molecular
iology or genetics

tarting salary within range
10,458–£13,527 per annum on the
A scale, plus USS

formal enquiries, and formal
pplications with curriculum vitae
nd the names of two referees, to
r J I Wray, Plant Molecular Gene-
ics Unit, University of St An-
rews, Sir Harold Mitchel Buil-
ing, St Andrews Fife, KY16 9TH
el 0334 76161 ext 7253) by 8
ecember 1989 (2129)A

UNIVERSITY OF DUNDEE
NINEWELLS HOSPITAL AND MEDICAL SCHOOL
DEPARTMENT OF BIOCHEMICAL MEDICINE

GRADUATE RESEARCH ASSISTANT
(EST/448/89/N)

& RESEARCH TECHNICIAN (GRADE 3 OR 2)
(EST/128/89/N)

Applications are invited from suitably qualified candidates for the above posts, tenable for 3 years and funded by the Sir Jules Thorn Charitable Trust The successful applicants will join a rapidly expanding research group studying the role of drug conjugating enzymes in susceptibility to adverse drug reactions Experience in molecular biology and/or protein purification would be an advantage, although not essential For the post of Research Assistant, applicants should possess at least a second class (upper division) Honours degree in a related subject The posts are available from January 1990, or as soon as possible thereafter

Salary for the Research Assistant on the RA 1B scale (£9816-£12381 p a) and for the Research Technician on the Grade 3 or 2 scale (£7254-£7711) Applications (in the form of full C V (2 copies) and the names and addresses of two referees) should be sent to, the **Personnel Office, The University, Dundee, DD1 4HN** Please quote appropriate reference numbers Closing Date 8 December 1989 Informal inquiries welcome to Dr M Coughtrie on (0382) 60111 Ext 2510

(2149)A

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nature

the widest international selection of jobs
in science — EVERY WEEK

AMERICAN CANCER SOCIETY
BASIC CANCER RESEARCH EMINENT SCHOLAR CHAIR
UNIVERSITY OF FLORIDA
(Edward R Koger Professorship)

Applications are invited for candidates to fill a faculty professorship in Basic Cancer Research, funded through an Eminent Scholar Chair sponsored by the American Cancer Society The candidate should have demonstrated a strong commitment to academic medicine Candidates must hold an Ph D , M D , or both, and have an accomplished research record in the investigation of fundamental processes of neoplasia A highly interactive individual would be desirable to take advantage of the research collegiality available at the University of Florida The scholar could be appointed in a basic science department or jointly with a clinical department Salary is negotiable and will be based on credentials Deadline for receipt of applications is January 15, 1990

Candidates for this position should be nominated or they should submit a letter of application stating teaching and research goals, and include a curriculum vitae and at least three names of reference to **Edward M Copeland, III, M D , Chairman of Professorship Search Committee, Department of Surgery, Box J-286, JHMHC, Gainesville, FL 32610**

The University of Florida is an equal opportunity, affirmative action employer (NW4634)A

UNIVERSITY OF CAMBRIDGE
Department of Applied Mathematics and Theoretical Physics
Silver Street, Cambridge, England CB3 9EW

Post Doctoral Positions in Gravitation and Cosmology

The Cambridge Relativity Group wishes to appoint a SERC supported Research Assistant with effect from 1 October 1990, for two years initially with the possibility of reappointment for one further year

The position will be in Cosmology or Quantum Gravity including string theory

Those interested should send a curriculum vitae and publications list to arrive no later than 15 December 1989 They should also arrange for at least two letters of recommendation from someone familiar with their work to arrive by the same date

Salary £10,458-£13,527 p a depending on age (2140)A

BIOCHEMIST POSTDOCTORAL POSITION

Study bacterial glycoproteins and the *Bacillus thuringiensis* insecticidal toxin Experience in HPLC and oligosaccharide characterization highly desirable Requires Ph D in Biochemistry or related area Salary $24,000 Two years expected duration Submit curriculum vitae and three references by Jan 2, 1990 to **Dr Kenneth W Nickerson, Biological Sciences, University of Nebraska-Lincoln, Lincoln, NE 68588-0118** AA/EOE

(NW4628)A

SIMON FRASER UNIVERSITY
CHAIR OF THE DEPARTMENT OF BIOLOGICAL SCIENCES

Applications and nominations are sought for the position of Chair of the Department of Biological Sciences at Simon Fraser University The Department has 35 faculty members whose teaching and research interests cover all the modern areas of Biological Sciences There is a graduate enrolment of some 160 students, and external research grant support in 1988/89 was $2 4 million

A Departmental Chair appointment is for up to five years and is renewable, the salary is negotiable The appointment would normally carry tenure Candidates should have an excellent record in research and a demonstrated ability as an academic leader

Applications or nominations should be forwarded, by January 15, 1990, to the **Dean of Science, Simon Fraser University, Burnaby, B C V5A 1S6**

This advertisement is directed to people who are eligible for employment in Canada at the time of application S F U offers equal employment opportunities to qualified applicants (NW4626)A

WESSEX REGIONAL CYTOGENETICS UNIT

SENIOR GRADE CYTOGENETICIST

A position for a Senior Cytogeneticist has become vacant in the above Unit The successful candidate will be expected to take considerable responsibility for the running of one of the three cytogenetic sections, in teaching junior members of staff and be prepared to rotate through all cytogenetic sections of the Unit Applicants will be expected to have the Diploma in Clinical Cytogenetics from the Royal College of Pathologists and to be fully conversant with all aspects of blood, solid tissue and prenatal cytogenetics Experience in leukaemia cytogenetics would also be an advantage

The Unit is comprised of a large diagnostic cytogenetic section and a well established molecular diagnostic section It actively pursues a number of research projects at the interface of cytogenetics and molecular genetics Salary scale £11,778 to £15,394

Enquiries to Dr Patricia Jacobs or Dr Nina Gregson — Tel (0722) 336212 ext 669 For an application form please WRITE to the **Personnel Department, Odstock Hospital, Salisbury, Wilts SP2 8BJ** Closing Date 15th December 1989 (2153)A

DEPARTMENT OF BIOCHEMISTRY

UNIVERSITY OF BRISTOL

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for a three-year appointment funded by the MRC The project involves the identification and characterisation of intramolecular signals required for faithful sorting/targetting/retention of integral membrane proteins specific to the trans Golgi network (TGN) Candidates should have experience in molecular and/or cell biology techniques However, lack of relevant experience should not discourage potential applicants The post is available from 1 1 90 on salary scale £12,879-£13,527 per annum

Informal enquiries can be made to Dr G Banting on Bristol (0272) 303030 ext 4915

For further details telephone Bristol 303136 (ansaphone after 5 00 p m) or write to the **Personnel Office, Senate House, Bristol BS8 1TH** Please quote reference A862

An equal opportunities employer

(2120)A

POSTDOCTORAL/ASSOCIATE RESEARCH SCIENTIST

positions available in the Department of Pharmacology at Yale University School of Medicine to study (1) mechanisms of action of drugs on hypoxic tumour cells with emphasis on the reductive activation of bioreductive alkylating agents, and (2) mechanism of drug induced differentiation of neoplastic cells Ph D required and experience in biochemistry and/or pharmacology desired Deadline for receipt of applications is January 31, 1990 Send curriculum vitae and three letters of reference to **Dr Alan C Sartorelli, Director, Comprehensive Cancer Center, Yale University School of Medicine, 333 Cedar Street, New Haven, Connecticut 06510**

Yale University is an Equal Opportunity/Affirmative Action Employer The Department of Pharmacology encourages applications from Women and Minority Group members (NW4622)A

UNIVERSITY OF OXFORD

DYSON PERRINS LABORATORY

CLONING OF ANTIBIOTIC BIOSYNTHETIC GENES

Applications are invited for an SERC funded postdoctoral research postion in the area of antibiotic biosynthesis in *Streptomyces*, The post is to start as soon as possible Salary will be in the RA1A range, £10458 to £12381, starting point depending on age and qualifications

Applicants should send a c v and ask two referees to write to **Dr A J Pratt, Dyson Perrins Laboratory, South Parks Road, Oxford, OX1 3QY** Oxford University is an equal opportunity employer (2157)A

UNIVERSITY OF NOTTINGHAM

PROFESSORIAL UNIT OF RESPIRATORY MEDICINE

Post-doctoral Research Worker required for an 18-month period with the possibility of extension The work is funded by the Cystic Fibrosis Research Trust and involves the development of micro-assay techniques for the 35 measurement of inflammatory factors including neutrophil granule products and tumour necrosis factor These methods will be applied to infants and children with cystic fibrosis in an attempt to identify independant markers of disease activity Experience with immuno-assay (ELISA or RIA) and of cell handling methods would be useful but not essential Salary on the Grade 1A scale from £10,458 per annum depending on age and experience

Enquiries to **Dr Dennis Shale, Respiratory Medicine Unit, City Hospital, Hucknall Road, Nottingham NG5 1PB or tele phone 0602 691169 ext 45781** (2123)A

UNIVERSITY OF DURHAM

DEPARTMENT OF ARCHAEOLOGY

IMAGING ARCHAEOLOGICAL STRUCTURES BY ELECTRICAL RESISTIVITY TOMOGRAPHY

(Re-advertisement)

Applications are invited for a Postdoctoral Research Assistant in Imaging Archaeological Structures by Electrical Resistivity Tomography for a research project funded by SERC in conjunction with Geoscan Research

Geophysical surveys now play a crucial role in archaeological conservation, excavation planning and resource assessment Existing techniques generally provide only 2-D area maps over buried features with minimal depth information This project will transfer recent developments in medical imaging technology to archaeology by developing a novel electrical resistivity tomography system capable of resolving archaeological structures in 3-D The research will involve theoretical studies, numerical simulation, laboratory and field experiments with interaction between the archaeological, medical and physical sciences Suitable candidates will have a strong background in applied mathematics, physics, geophysics or related fields

The appointment is tenable for three years from as soon as possible and the initial salary will be on the Research Staff Scale Grade 1A (£10,458-£12,381 p a) Informal enquiries may be made to Dr M Noel, Department of Archaeology, (091) 374 3631

Further particulars (quoting reference SS/AS/89 04) may be obtained from the Registrar and Secretary (Faculty of Social Sciences), University of Durham, Old Shire Hall, Old Elvet, Durham DH1 3HP, telephone (091) 374 2995, to whom applications (three copies), including the names and addresses of three referees, should be sent not later than Wednesday 6 December 1989 Previous candidates need NOT apply (2074)A

PHLS CENTRE FOR
APPLIED MICROBIOL
& RESEARCH

DIVISION OF BIOLOGIC

EUROPEAN COLLECT
OF ANIMAL CELI
CULTURES

BASIC GRADE
MICROBIOLOGIST

Applications are invited fo
above post within the Eur
Human Cell Bank section c
Collection The function o
Collection is to store and ch
terise cell lines, contract
culture and to act as an
nationally recognised P
Depository The Euro
Human Cell Bank is involved
a specific collection of Huma
lines derived from patients
genetic disorders and chr
some rearrangements Dutie
include the generation of
transformed lymphoblastoid
lines from peripheral
samples, cell culture and
preservation The post
involves extensive liaison
Clinical Geneticists throug
the UK There may also b
opportunity for the trainin
molecular biological techn
being developed with the c
tion for cell line characterisa

A graduate scientist is so
preferably with cell culture e
ience but this is not essential a
training will be given

Salary scale is from £7
£11072 depending on qual
tions and experience (u
review) The post is init
funded for two years and Nat
Health Service Terms and Cc
tions will apply

The post may be discussed i
mally with Dr B J Bolton,
phone (0980) 610391 ext 2
2512

Application forms can
obtained from the Personnel
cer, PHLS Centre for Ap
Microbiology and Res
Porton Down, Salisbury, W
SP4 0JG, telephone (0980) 61
ext 2240, to whom compl
forms should be returned by
December 1989 quoting
number 0507 (2146)

POSTDOCTORAL POSITION

available immediately for a Ph D in physical chemistry/biochemistry to study the mechanism of electron transfer reactions of the membrane protein cytochrome P-450 Experience with enzyme kinetics and/or stopped-flow spectrometry helpful Salary negotiable Send C V and 3 references to **Dr L Waskell, M D , Ph D , VA Medical Center, University of California, San Francisco, 4150 Clement Street, San Francisco, CA 94121** Equal Opportunity Employer/Affirmative Action

(NW4638)A

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Still first with the best in science

DIVISION DIRECTORS

DIVISIONS OF GASTROENTEROLOGY, INFECTIOUS DISEASES & NEPHROLOGY

The Department of Medicine of the University of Toronto Faculty of Medicine is seeking Directors for the following divisions — Gastroenterology, Infectious Diseases & Nephrology These positions are full-time appointments with academic rank of either Associate Professor or Professor as appropriate *Responsibilities will include* the coordination of clinical services among the University teaching hospitals, the organization and supervision of under*graduate medical education and* the postgraduate training program, and the fostering of research programs Candidates should have an established reputation of clinical expertise and research accomplishment The successful *candi*dates will *be* expected to continue personal programs of research Candidates must have an F R C P (C) or equivalent and be eligible for licensure in the province of *Ontario* The *Uni*versity of Toronto encourages applications from qualified women and men, members of visible minorities, aboriginal *people and persons with disabili*ties In accordance with Canadian immigration requirements, this advertisement is directed firstly to Canadian citizens and permanent *residents* Closing date for applications is February 23, 1990

*Ap*plications should include the candidate's curriculum vitae and be addressed to

Arnold Aberman, M D
Chairman,
Dept of Medicine
University of Toronto
Faculty of Medicine
Suite BW1-628
585 University Avenue
Toronto, Ontario, Canada
M5G 2C4

(NW4625)A

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nature

Still first with the best in science

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF HUMAN ANATOMY AND CELL BIOLOGY

NEUROBIOLOGY GROUP

POSTDOCTORAL RESEARCH ASSOCIATE

Initial salary will be up to £12,879 p a

This is a three year MRC-funded post to investigate the mechanism of neurite outgrowth The project will focus on two surface glycoproteins linked to the membrane by a glyosylphosphatidylinositol group You will join an enthusiastic and expanding group which uses a variety of cell and molecular biological techniques Experience of these techniques and/or lipid carbohydrate analysis is preferred

Informal enquiries to Dr Diana Moss, Department of Biophysics, King's College, London on 01-836 8851

Quote ref REV/540/NAT

Closing date 20th December, 1989

Applications, by c v with the names of three referees, should be sent to - The Director of Staffing Services (AS), The University, P O Box 147, Liverpool L69 3BX, from whom further particulars may be obtained

An Equal Opportunity Employer (2177)A

PUBLIC HEALTH LABORATORY SERVICE BOARD

EXETER PUBLIC HEALTH LABORATORY

FOOD RESEARCH DIVISION

MEDICAL LABORATORY SCIENTIFIC OFFICERS (GRADE 1)

Salary £7,415-£10,148
per annum

Applications are invited for two MAFF funded posts (2 year fixed term)

One post would involve research into the effects of storage on Salmonellas in eggs, the other viral contamination of molluscan shellfish

Applicants should have experience in working with pathogenic microorganisms and an interest in Food and Environmental Mircrobiology

Application forms and job descriptions from Dr T J Humphrey, Food Research Division, Public Health Laboratory, Church Lane, EXETER EX2 5AD

Telephone Exeter (0392) 402953

Closing date 1st December 1989 (2147)A

平成2年度 基礎科学特別研究員制度の案内について

科学技術庁と理化学研究所とは 連携して我が国の基礎研究を強力に推進するため 平成元年度に引続き平成２年度の基礎科学特別研究員の募集を計画しています。斬新な研究課題を自主的に遂行できる若い在外の我が国研究者の応募を期待します。

1 採用予定人員／25名

2 受 入 機 関／理化学研究所

3 募 集 分 野／物理学 化学 生物学 工学の各分野で 理化学研究所で実施可能な研究。

4 対　象　者／原則として平成２年４月１日現在35才未満の健康な者で 博士号取得者
又はこれと同等の研究能力を有すると認められる者。

5 待　遇　等／(但し 平成元年度実績)
1 謝 金 月額 475千円程度(社会保険料 税込)
2 通勤費 実費 (上限26千円／月)
3 住宅費 家賃の一部支給
以上のほか 研究費として1 360千円／年程度。

6 契約予定期間／通算３年間を限度とし 毎年度所要の評価により契約更新。

7 応募の意向のある方は12月15日金までに下記にお問い合わせ下さい。

1 科学技術庁 科学技術振興局 研究振興課
〒100 東京都千代田区霞が関２丁目２番１号
電話 03-581-5271 内線532(直通 03-503-0013)
FAX 03-581-7033

2 理化学研究所 研究業務部 基礎科学特別研究員担当
〒351-01 埼玉県和光市広沢２番１号
電話 0484-62-1111 内線2451 2452(直通 0484-63-3687)
FAX 0484-62-1554

8 そ　の　他／本制度は関係予算の成立を前提としており その事情により変更がありえますのでその旨御承知おき下さい。 (W6750)A

科学技術庁／理化学研究所

FACULTY-LEVEL RESEARCH POSITION

available for studies on cell-matrix interactions involving extracellular matrix proteins and protein-based polymers (including elastomeric polypeptide matrices), chemotaxis and cell adhesion Faculty level and salary commensurate with experience Interested parties please contact **Dr Dan W Urry, Director, Laboratory of Molecular Biophysics, School of Medicine, University of Alabama at Birmingham, P O Box 300, Birmingham, AL 35294** Telephone 205-934-3543 *An Equal Opportunity/Affirmative Action Employer* (NW4633)A

Nitric Oxide Postdoctoral Position

available immediately to study the metabolism and function of NO in the cardiovascular system Cell culture and mass spectrometry will be used

Send CV and name of two references to **Dr Jurgen Schrader, Physiol Dept , Univ Dusseldorf, Moorenstrasse 5, D-4000 Dusseldorf** (W6748)A

FELLOWSHIPS

LISTER INSTITUTE RESEARCH FELLOWSHIPS

30 October, 1989

Applications are invited for Lister Institute Research Fellowships, of which up to five will be available from about October 1990, each tenable for five years An extension would be considered in appropriate circumstances, subject to satisfactory review Candidates must be post-doctoral in the bio-medical field and aged under 34 years, with evidence of independent research, including at least two years' post-doctoral research, and with an interest in a scientific aspect of preventive medicine *Special arrangements will be considered for those clinicians who are required to undertake further training for accreditation in their speciality* A permanent post temporarily vacated by the Fellow must be protected by his/her employing institution which will be expected to appoint a temporary replacement during the period of the Fellowship The salary, payable by the employing institution and reimbursed by the Institute, will normally be on University clinical or non-clinical scales, with London Weighting if appropriate, plus USS and N I contributions A generous allowance for consumables and minor equipment is also payable

Note In exceptional circumstances, as for example where there have been career breaks on family or other grounds, the Institute would consider electing Fellows aged up to 40 years Personal details supporting the claims of such candidates must be provided in writing The Institute will then give a decision as to whether or not the candidates may proceed to submit a full application for a Fellowship

Application forms and further particulars may be obtained from — **The Secretary, Ref N/I, The Lister Institute of Preventive Medicine, Brockley Hill, Stanmore, Middlesex HA7 4JD** to whom completed forms must be returned not later than 26 January, 1990 (2134)E

The Wellcome Trust

Advanced Training Fellowships in Toxicology

The Wellcome Trustees wish to strengthen the quality of toxicological research in universities In particular, they hope to promote the career development of men and women who wish to pursue research on toxicological problems in departments not only of pharmacology and toxicology, but also departments of physiology and other related subjects

The Trustees therefore announce a limited number of advanced training fellowships in toxicology Applications are invited from medical and post doctoral scientific graduates who have worked in universities, institutes or hospitals in the United Kingdom or the Republic of Ireland for at least the past three years, or who did so before taking up an appointment overseas and who now wish to return from abroad These awards, which are tenable for up to three years, are intended for candidates who are able to provide evidence of their ability to undertake original research, and who wish to extend their research experience The fellowship will be held in an appropriate department in a British or Republic of Ireland university or research institute, but part of the fellow s time may be spent in an appropriate department overseas

Applications will be particularly welcome from men and women whose previous experience has not been in toxicology, but who have been trained in one of the related scientific disciplines, and who now wish to develop a career in toxicology Candidates are welcome to discuss their plans with the scientific staff of the physiology and pharmacology section of the Trust

Candidates should preferably be less than 32 years of age for science graduates and 34 for medical graduates However, equal consideration will be given to those over this age where their career has been affected either by a late start, or by interruption for personal or family reasons Applicants must be nominated by the head of department in the university or institute in which the candidate proposes to work Salary, according to age and experience, will be based on the Trust's own fellowship scale which is related to the university lecturer scale

Application forms can be obtained from
The Grants Section (T/F), The Wellcome Trust,
1 Park Square West, London NW1 4LJ Tel 01-486 4902

Applications must be returned by 23rd February 1990
Late applications will not be considered

(2127)E

Faculty of Science

Postdoctoral Research Fellow in Cellular Mechanisms of Memory

Applications are invited from candidates with a good background in neurochemical techniques for a SERC-funded postdoctoral fellowship in cellular mechanisms of memory The research will be carried out in the Biology Department at the Open University campus, Milton Keynes, under the supervision of Professor Steven Rose and Dr Michael Steward of the University's Brain and Behaviour Research Group The project concerns the role of neurotransmitter release, protein phosphorylation and receptor-second messenger ion channel coupling in memory consolidation processes in the young chick

Applicants should have a PhD degree in a relevant subject area

The post is available immediately for a period of three years Salary will be either on the Grade 1A scale for Research and Analogous Staff (£10,458–£16,665 p a), or the Grade II scale (£15,372–£20,469 p a), according to age and experience

Application forms and further particulars are available from **Ann Hall (7107/), Faculty of Science, The Open University, Walton Hall, Milton Keynes, MK7 6AA**, or telephone Milton Keynes (0908) 655938 there is a 24-hour answering service on 653868

Candidates may also wish to contact Professor Rose on (0908) 652125 to discuss the project

Closing date for applications 13 December

Equal Opportunity is University Policy (2176)E

The Open University

POLYTECHNIC SOUTH WEST
Faculty of Science
Department of Environmental Sciences
Plymouth

Research Fellowship in Organic Chemistry

Salary within the range £10,575 to £13,914 per annum (starting point dependent upon qualifications and experience)

Applications are invited for a Research Fellowship to undertake a 3 year project under the supervision of Dr Steven Rowland, entitled "A quantitative and qualitative characterisation of organic matter from a variety of recent sedimentary environments" The project will involve a multi-method approach to the characterisation of humic substances and "protokerogen" and is funded by a major international oil company

Applicants should possess, or have submitted, a PhD in some aspects of organic geochemistry and preferably have experience in pyrolysis techniques The appointment will commence as soon as is mutually agreeable after 1st January 1990

Application forms, to be returned by Friday 15th December 1989, and further details are available from the Personnel Department, Polytechnic South West, Drake Circus, Plymouth PL4 8AA, or by telephoning (0752) 232167 (2166)E

andoz Ltd. is offering a

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or the development of a novel adjuvans system on the basis of a controlled
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ither a biochemical, immunological background or training in field of
ontrolled release are prerequitite. Expertise in the development of vaccines
tetanus) would be helpful.

pplications, including curriculum vitae, should be sent to:

SANDOZ

Sandox Ltd., Personnel Department, ref. 9808,
attention Mr. R. Zbinden,
CH–4002 Basle, Switzerland

SANDOZ

(W6736)E

NIVERSITY OF KEELE

Department of Biological Sciences

ESEARCH FELLOWSHIP IN CELL BIOLOGY

plications are invited from hemists and cell biologists for wo-year Research Fellowship he Department of Biological ences The appointee will work Professor J B Lloyd in the llular Pharmacology Research oup on a project jointly funded SERC and ICI Pharmaceutic- , investigating the cellular dling of novel biodegradable yesters being developed as ential drug-carriers Initial ary up to £12,381 with USS

urther details and application ms from the Registrar, Univer- of Keele, Keele, Staffs, ST5 G, (Tel (0782) 621111 Ext, 8) Closing date 15 December 9 (2124)E

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Sabbatical and Postdoctoral Fellowships

Keratinocyte Biology

State University of New York
at Stony Brook
Department of Oral Biology
and Pathology
School of Dental Medicine

The aim of these fellowships is to foster research in keratinocyte biology in areas relevant to epithelial gene therapy Emphasis is placed on secretory activity of epidermal keratinocytes although other topics of research will be considered Stipend level and duration is negotiable At the sabbatical level, stipend is intended to supplement an existing salary

Send résumé, brief summary of research interests, copies of recent publications and names of references to **Dr Lorne B Taichman, Professor, Department of Oral Biology and Pathology, School of Dental Medicine, SUNY Stony Brook, Stony Brook, NY 11794-8702** (NW4639)E

FELLOWSHIP

LABORATORY OF NEUROSCIENCES

The Laboratory of Neurosciences in the National Institute on Aging, National Institutes of Health, Public Health Service, invites applications for a Fellowship position to study the neurobiology of genes from human chromosome 21 and mouse chromosome 16 by the use of transfected cultured neurons and the development of transgenic mice Current laboratory projects include the effects of human trisomy 21 (Down Syndrome) and mouse trisomy 16 (a model of trisomy 21) or ionic currents, gene expression, lipid metabolism and receptor-second messenger signalling Candidates should have experience in molecular biology and interest in the isolation of genes and chromosomal segments with the aim of determining the role of specific genes in aneuploid-related neurophysiological abnormalities Available from late 1989 - early 1990 Position is located in Bethesda, Maryland and salary is commensurate with qualifications Send resume and the names of three references to

Stanley I Rapoport, M D
Chief, Laboratory of Neurosciences
National Institute on Aging
Bldg 10, Room 6C-103
9000 Rockville Pike
Bethesda, Maryland 20892
U S A

NIH IS AN EQUAL OPPORTUNITY EMPLOYER

(NW4532)E

Ciba-Geigy Biotechnologie Department Basel, Switzerland

A Postdoctoral Fellowship Position

is immediately available for a period of up to three years at the Biotechnologie Department of Ciba-Geigy to study the molecular interactions between a protein and its receptor The project will involve both genetic engineering of the cloned ligand protein and immunological epitope mapping of the binding domain to the receptor

Applicants must have a good working knowledge of molecular biology techniques, expertise in immunological and biochemical characterization of ligand-receptor complexes

The successful candidate is encouraged to establish contacts with the academic community This position offers good possibilities to develop own ideas in a stimulating research environment Publication of results is strongly encouraged.

Applications, including a curriculum vitae, a description of current research interests and the names and addresses of three referees should be forwarded under ref "Nature 2731" to Mr H. Schmid, CIBA-GEIGY Limited, Personnel Department, P O Box, 4002, Basle, Switzerland

CIBA–GEIGY

Offen für Ihre Einstellung. (W6733)E

THE UNIVERSITY OF BIRMINGHAM
SCHOOL OF BIOLOGICAL SCIENCES
M.R.C. POSTDOCTORAL RESEARCH FELLOWSHIP

Applications are invited for a three year Postdoctoral Research Fellowship in the laboratory of Dr Chris Thomas to study the molecular biology of the processes which lead to partitioning of bacterial plasmids to daughter cells prior to cell division The work will involve a range of techniques in molecular genetics and protein biochemistry, experience in these areas is highly desirable Salary will be in the Research Fellow IA range (£10,458-£16,665) and the post is tenable from 1 January 1990

Informal enquiries to Dr Chris Thomas by phone (021 414 5903)

Further particulars and application forms can be obtained from the **Director of Staffing Services, the University of Birmingham, Edgbaston, Birmingham B15 2TT** (telephone 021 414 6483 — answerphone) to whom applications (3 copies) should be returned by 15th December quoting reference number 13009

AN EQUAL OPPORTUNITIES EMPLOYER

(2168)E

RESEARCH FELLOWSHIPS IN THE ENVIRONMENTAL SCIENCE

Applications are invited from outstanding, young research workers for a limited number of Post-Doctoral and Advanced Research Fellowships, located at various British Universities, Polytechnics and Research Institutes

All candidates should be able to show an aptitud for original and independent research and must posses a PhD Advanced Fellowship applicants should also hav relevant post-doctoral experience and are expected to b of the calibre of new blood lecturers

Fellowships will be tenable for a period of up to 5 years in the first instance Salary will be in the range **£10,458** to **£16,665** (as at 1 4 89)

Proposals for Fellowships, together with application forms RF1 and RF2, are available from the Registrar's Offices and should be submitted to NERC by 15th December 1989

For further details please contact Dr Colin Vincent, Higher Education Section, NERC, Polaris House, North Star Avenue, Swindon SN2 1EU or telephone Swindon (0793) 411512

**Natural
Environment
Research
Council**

(2180)

POST-DOCTORAL FELLOWSHIP IN VIROLOGY

is funded to investigate the immunologic and pharmacologic mechanisms controlling herpes simplex virus reactivation in anima models Experience in Molecular Biology and Immunology preferred Opportunity to work with a team of molecular virologists, immuno logists, and pharmacologists interested in this area Post-Doctoral salary as per NIH guidelines Curriculum Vitae, three letters of reference and statements of research goals and interests to **Dr Jay S Pepose, Departments of Ophthalmology and Pathology-Box 8096, Washington University School of Medicine, 660 S Euclid Avenue, St Louis, MO 63110** (NW4613)E

UNIVERSITY OF CONNECTICUT HEALTH CENTER
DEPARTMENT OF PEDIATRICS

Postdoctoral Research Fellow Position

We are seeking applicants for a post-doctoral fellowship in the area of human molecular genetics Requirements include training and experience in molecular biology Experience in gene cloning, characterization of mutations and DNA sequencing will be an advantage A variety of research projects in Connective Tissue, Eye disorders and schizophrenia are currently in progress We have excellent clinical and laboratory facilities and offer a competitive salary

The applicants should submit a complete C V , including names of three references to **Petros Tsipouras, M D , Department of Pediatrics, University of Connecticut Health Center, 263 Farmington Ave , Farmington CT, 06032**

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FELLOWSHIPS continued

ostdoctoral Fellowship Position

olecular Immunology and Virology

postdoctoral position is nediately available for the dy of either the molecular logy of HTLV-1 and HIV-1, icture-function relationships the human interleukin-2 eptor or DNA binding pro- is that regulate transcription T cell activation genes Prior)erience in molecular bio- y and/or biochemistry is uired

erested applicants should id curriculum vitae and mes of three references to **Dean W Ballard, c/o Dr rner C Greene, Howard ghes Medical Institute, ke University Medical Cen- , P O Box 3037, Durham, rth Carolina 27710** or Tele- 919-684-5458

(NW4567)E

ASSISTANTSHIPS

UNIVERSITY OF BRISTOL
EPARTMENT OF PHYSIOLOGY

RESEARCH SSISTANTSHIP

)plications are invited for Research Assistantship st (Grade 1A or 1B) avai-)le immediately for up to o years This post is fun- d by a MRC Programme ant to Dr F Cervero for earch into the mechan- ns of somatic and vis- ral sensation Applicants this post should have perience in either electro- ysiology of the mamma- n peripheral or central rvous system or in imunohistochemistry

formal enquiries may be ade to Dr Cervero on istol (0272) 303464 or 3030 ext 4464/4856 Fax)3497

)r further details tele- ione Bristol 303136 nsaphone after 5 00 p m) write to the **Personnel ffice, Senate House, Bris- l BS8 1TH** Please quote ference A861

osing date for applica-)ns is 7th December 1989

n equal opportunities nployer (2148)P

STUDENTSHIPS

ROYAL HALLAMSHIRE HOSPITAL, SHEFFIELD

University Department of Surgery

Applications are invited for a Research Studentship, tenable for a period of two years with a possible extension of a further year Applicants should possess an Honours Degree in Biomedical Sciences The work is funded by the Special Trustees of the Former United Sheffield Hospitals and will investigate the detection of liver tumours by radioisotope scanning and also the role of tumour cells in metastatic disease This will involve the techniques of isotope imaging and FACS analysis

Applicants should send a curriculum vitae with the names of two academic referees to the Personnel Department, Westbrook House, Sharrow Vale Road, Sheffield S11 8EU, not later than 8 December 1989

Informal enquiries may be made to Mr G Jacob, University Department of Surgery, Royal Hallamshire Hospital, telephone number Sheffield (0742) 766222, ext 2163 (2165)F

Research Studentship in Neuropharmacology

Department of Physiology and Pharmacology Nottingham University Medical School

A 3 year studentship sponsored by Roussel Laboratories Ltd to work on "*In Vivo* studies on central acetylcholine release *mechanisms in relation* to the development of novel drug treatment of cognitive disorders" to work with Professor C A Marsden and Dr G W Bennett is available immediately Candidates should hold at least an upper second class degree Applications should be sent to **Dr G W Bennett, Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH** (Tel 0602 709475 for further information) to include a curriculum vitae and the name, address and telephone number of two academic referees (2122)F

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INSTITUTE OF PSYCHIATRY

(University of London)

Department of Psychology

Research Studentships in Psychology and Neuroscience

Applications are invited from students wishing to read for a doctorate The Department has facilities for research in a wide selection of fields, ranging from studies of the neuroanatomical, neurochemical and molecular bases of behaviour in animals to the clinical investigation of abnormal human behaviour in a variety of psychiatric and neurological disorders Wherever possible fundamental and clinical research programmes are closely co-ordinated There is extensive collaboration with other Departments (eg Neuroscience, Neuropathology, Psychiatry, Neurology) in the Institute of Psychiatry Graduate students currently in the Department are supported by Studentships from a variety of sources, including the pharmaceutical industry (Glaxo, Shering) *Awards are made on a competitive basis* Applicants from the UK should have or expect to gain at least an Upper Second Class Honours degree in psychology or neighbouring fields within the biological, social or medical sciences Applicants from overseas should be of equivalent standard with a good command of English

Applications should be sent as soon as possible (and not later than 16 February 1990) to **Mrs Shirley Chumbley, Department of Psychology, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF** (from whom application forms can be obtained) (2141)F

SCHOOL OF PHARMACY UNIVERSITY OF LONDON

UNIVERSITY/INDUSTRY CENTRE FOR PHARMACEUTICAL RESEARCH

Postgraduate Studentships in Pharmaceutical Sciences

Applications from students trained in chemistry, biochemistry, pharmacy or pharmacology are invited for three Ph D studentships in any of the following areas

(1) Receptor and Ion-Channel Biochemistry
(2) Glycerol and Sphingolipid Biosynthesis and Degradation
(3) Synthetic and/or Biological Studies of novel transmembrane delivery
(4) CNS, Anti-inflammatory and Anaesthetic drug design and synthesis

The Centre is equipped for all aspects of the above research programmes Stipends will be in the range of £4,600 to £6,250 depending upon the source of funding and the experience of the candidates suitability Applications should be sent to **Professor William A Gibbons, School of Pharmacy, 29-39 Brunswick Square, London WC1N 1AX** from *whom further particulars may be obtained* (2142)F

Prize Studentships/ Research Scholarships

DEPARTMENT OF BIOCHEMISTRY UNIVERSITY OF DUNDEE

Able candidates wishing to study for the degree of Ph D are sought for nomination to the above positions The Department is one of the most successful *and highly rated in the UK* for biochemistry research and is very well equipped and funded In addition to the outstanding research facilities and working environment, access to outdoor recreational facilities is unrivalled and accommodation/living expenses are among the lowest in Britain Research projects are available in a wide range of areas including cell biology, molecular genetics, protein phosphorylation, intracellular signalling and cell cycle control

Applications, including a full curriculum vitae and the names and addresses of two referees should be sent as soon as possible to **Dr M J R Stark, Department of Biochemistry, The University, Dundee DD1 4HN** (tel 0382–23181 ext 4250) Prospective applicants are requested to phone Dr Stark immediately for a copy of the Departmental brochure and information about the Tayside area Travel and overnight accommodation will be provided for applicants invited for interview (2178)F

COURSES & CONFERENCES / WORKSHOPS

Natural Environment Research Council

NATO ASI Series

MOLECULAR TECHNIQUES IN TAXONOMY

UEA NORWICH

JOHN INNES INSTITUTE

AFRC Institute of Plant Science Research

NATO Advanced Studies Institute
Norwich UK 9–20 July 1990

This **tutorial course** aims to bring together some 20 international authorities and 70 young researchers to discuss and learn about this exciting interface Lectures, seminars and laboratory practicals will use molecular and computer methods for systematic studies

Topics will include — Taxonomic concepts, DNA hybridization, PCR and DNA sequencing, mtDNA, rDNA, scDNA, RFLPs, Sequence databases, Phylogenies, Computer programmes, Non-DNA techniques, Rapid diagnosis Applications to microbes, plants, insects and vertebrates — in Agriculture, Conservation, Medicine and Evolution

Speakers will include — Humphrey Greenwood (British Museum), James Murray (Virginia), Hampton Carson (Hawaii), Jean David (Gif), Eleftherios Zouros (Crete), Christine Simon (Hawaii), Jeffrey Powell (Yale), Dieter Sperlich (Tubingen), Robert Cedergren (Montreal), Jeffrey Doyle (Cornell), Michel Solignac (Gif), Martin Kreitman (Princeton), Antonio Fontdevilla (Barcelona), Bryan Clarke (Nottingham), David Swoffard (Champaign), Peter Young (Norwich), and others

The Location is the University of East Anglia which, along with several Research Institutes, is set in parkland on the edge of the ancient City of Norwich

Applications to Professor Godfrey Hewitt University of East Anglia, Norwich NR4 7TJ before **31 January 1990** should include a CV with research interests — molecular or taxonomic expertise is not a prerequisite Full accommodation fee is £390, please explain in your application if you could not attend without a subsidy Some NATO countries give grants for these ASIs (2172)C

FIRST ANNOUNCEMENT

ORAL IMMUNISATIO
USING RECOMBINAN
BACTERIA

June, 6-7, 1990, Munich, FR

Speakers include B Askonas, D Clements, J Clark-Curtiss, Curtiss, G Dougan, P Freimu T L Hale, M Levine, J Hol gren, J B Kaper, S Kaufma J J Mekalanos, M Radm P Reeves, M H V Regenmort W S Robinson, J C Sad J Sanchez, S Snapper, B A Stocker, R Weiss

Organisers M Hofnung, Ins tut Pasteur, Paris, F Schod Max-Planck-Institut fur Bi chemie, Martinsreid

Supported by the Walt Schulz Foundation and the C Friedrich v Siemens Foundatio

20 abstracts will be accept for poster presentations Dea line for submission March, 1990

Information and abstract su mission F Schodel, Ma Planck-Institut fur Biochem D-8033 Martinsreid, Tel 8578 3759, Fax (89) 8578 3777 (W6744)C

NATO Advanced Research Workshop
Phytochrome Properties and Biological Action
Chichester, England: July 22–27, 1990

The meeting forms part of the NATO Cell Signalling series and will deal in detail with the mechanisms by which phytochrome regulates plant development Topics to be covered include — Molecular properties of phytochromes, Intracellular location, biogenesis, and turnover of the phytochrome molecule Signal transduction mechanisms Regulation of gene expression by light Evolution and biological function of phytochrome Phytochrome expression in transgenic plants The proceedings of the meeting will be published as part of the NATO ASI series

Those wishing to attend the workshop should write to the conference organiser including a short Curriculum vitae and an abstract (50–100 words) of their proposed contribution Deadline for applications is DECEMBER 31ST 1989

It may be possible to give financial assistance to some selected applicants, especially those from Greece, Portugal and Turkey Scientists from these countries are particularly encouraged to apply

For further information contact the conference organiser, **Dr Brian Thomas, AFRC — IHR, Worthing Road, Littlehampton, West Sussex BN17 6LP U K** Tel 0903-716123 FAX 0903-726780 (2163)V

EMBO Course 1990

Development, regeneration and plasticity of the nervous system — an introductory experimental course

Max-Planck-Institut fur Entwicklungsbiologie Tubingen

February 18 – March 3, 1990

Deadline for application January 10, 1990

15 participants

Topics

Plasticity of synaptic connections in the central nervous system Genes involved in neurogenesis during embryonic development of Drosophila Retinogenesis in vitro — Axonal growth in retina organ culture — Cell adhesion molecules In vitro studies — Axonal growth and guidance Ocular-dominance plasticity in developing kitten visual cortex An analysis of cell lineage in the rat cerebral cortex An analysis of cell lineage in the rat cerebral cortex using a retroviral vector Shifting connections in the retinotectal system Influences of peripheral nerve-derived factors on CNS regeneration Experimentally induced neural projection during embryogenesis of the chick visual system

Applicants should provide a brief c v and reasons for their wish to participate (not more than 1 page)

Applications should be sent to **Drs F Bonhoeffer & U Schwarz, MPI fur Entwicklungsbiologie, Spemannstr 35, D-7400 Tubingen, FRG,** Tel 07071/601 340 or 601 413 Fax 07071/601 300 (W6743)C

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United Nations Industrial Development Organization

INTERNATIONAL CENTRE FOR GENETIC ENGINEERING AND BIOTECHNOLOGY (ICGEB)

MOLECULAR AND GENETIC APPROACHES TO PLANT STRESS

15–17 February 1990, New Delhi, India

Invited speakers and moderators include.

M Bhattacharyya (St Louis, USA), F R Bidinger (ICRISAT, India), C -W Chi (Shanghai, PR China), Y -L Fan (Beijing, PR China), A Gatehouse (Durham, UK), T K Hodges (West Lafayette, USA), R Hull (Norwich, UK), R Johnson (Pullman, USA), K Kloppstech (Hannover, FRG), J Leach (Manhattan, USA), J Manners (Brisbane, Australia), J Mullet (College Station, USA), K S Nwanze (ICRISAT, India), H Pakrasi (St Louis, USA), J Peacock (ICRISAT, India), R S Rana (New Delhi, India), M A Rezaian (Adelaide, Australia), E.A Siddiq (Hyderabad, India)

Sessions will cover

Plant viruses, fungal diseases, pest resistance, insecticidal proteins, plant transformation and regeneration, RFLP analysis, stress-regulated gene expression, genetic variation in pathogens and pasts, heritability of resistance to abiotic stress, herbicide degradation and resistance Two poster sessions will allow participants to present their work

Registration

The Symposium will be held at the Ashok Hotel, New Delhi, with registration on Wednesday, 14th February

Aims

The Symposium will bring together molecular biologists and geneticists concerned with biotic and abiotic stress in plants Lectures and discussions will focus on the use of molecular, cellular and genetic techniques to accelerate the breeding of plants with enhanced stress resistance Since ICGEB has a mandate for research and advanced teaching in genetic engineering and biotechnology for the benefit of developing countries, applications are especially welcome from citizens of these countries A limited number of travel scholarships are available for applicants from ICGEB member countries

Application

The deadline for applications is 14th December 1989 Applications may be made by post or by fax and should include a curriculum vitae, a brief description of research interests, and a fax or telex number for reply The organizers will notify those who have been accepted as soon as possible after the deadline The total number of participants will be limited to about 100 Applications should be addressed to **Mr. G Chatterjee, Administrative Officer, ICGEB, NII Campus, Shaheed Jeet Singh Marg, New Delhi-110067, India (fax: 91-11-6862316)** Successful applicants will be expected to present a paper (W6453)M

UNIDO

Genetics Short Course

16-27 July 1990

1st Annual Short Course in Medical & Experimental Mammalian ienetics Conducted by Johns Hopkins University and The Jackson aboratory at Bar Harbor, Maine Morning and evening lectures overing the field are supplemented by workshops, tutorials, and emonstrations Inquiries, applications to

H Roderick, Ph D, c/o Training & Education Office The Jackson boratory, 600 Main St, Bar Harbor, ME 04609, or V A McKusick, .D, 1007 Blalock, Johns Hopkins Hospital, Baltimore, MD 21205

(NW4619)C

MPSA

Eighth International Conference on Methods in Protein Sequence Analysis

Kiruna, Sweden, July 1–6, 1990

en sessions on Methodology and instrumentation, Sample preparation, Proteolysis, Modified residues and chemical problems, Synhetic peptides, Mass spectrometry, Synergism with DNA analysis, ynergism with 3D analysis, Comparisons and data banks, Predicions and interpretations

ectures, discussions, posters, and equipment

or information and application forms

Hans Jornvall
Department of Chemistry I
Karolinska Institutet
S-104 01 Stockholm, Sweden

(W6751)C

THE NEW YORK ACADEMY OF SCIENCES

Conference on:

Central and Peripheral Significance of Neuropeptide Y and Its Related Peptides

Baltimore Hyatt Inner Harbor, Baltimore, Maryland

April 2-4, 1990

The isolation and characterization of Neuropeptide Y (NPY) in the early 1980's has stimulated a number of studies into the molecular biology, biochemistry and physiology of this neuropeptide and its related peptides The ubiquity of NPY in the brain and periphery suggest that this neuropeptide is of physiological importance in homeostatic regulatory mechanisms of a number of species This conference will provide a forum for investigators to exchange ideas on the structural, functional and anatomical aspects of NPY with the hope of elucidating strategies for the development of selective ligands for the NPY receptors which could potentially unravel the biological and clinical significance of NPY in the nervous and cardiovascular systems

There will be contributed poster sessions in conjunction with this conference *Posters are encouraged* The deadline for submission of abstracts is *February 1, 1990* The entire abstract, including title, author(s), and affiliations, must be typed single-spaced and contained within a rectangle that measures 5 × 4 and 3/6 inches (W × L) Abstracts should be sent to one of the chairpersons (Abstract form is not necessary)

CONFERENCE CHAIRPERSONS

Dr Janet M Allen, Dept of Medicine, Royal Postgraduate Medical School, London W12 0NN, England

Dr James Koenig, Laboratory of Neuroendocrinology, Massachusetts General Hospital, 149 Thirteenth Street, Charlestown, MA 02129, USA

For further information contact **Conference Dept, The New York Academy of Sciences, 2 East 63rd Street, New York, NY 10021, USA TEL 212-838-0230, FAX 212-888-2894** (NW4637)C

COURSES & CONFERENCES

EUROPEAN MOLECULAR BIOLOGY ORGANIZATION
PRACTICAL COURSE

"GENETIC MANIPULATION OF *STREPTOMYCES*"

JOHN INNES INSTITUTE, NORWICH, ENGLAND
14–28 July 1990

The course will include gene cloning, protoplast fusion, mating, phages and plasmids Instructors M J Bibb, C J Bruton, M J Buttner, K F Chater, D A Hopwood, H M Kieser, T Kieser, R E Melton and J White

Further particulars and application forms from **Professor D A Hopwood, John Innes Institute, Colney Lane, Norwich NR4 7UH, England** (Deadline for applications 1 April 1990) (2159)A

HEART RESEARCH

The Coronary Artery Disease Research Association supports investigations into the non-invasive, presymptomatic diagnosis of cardiovascular disease, a fuller understanding of atherogenesis and the evaluation of measures to arrest/protect against the disease process with the aim of preventing premature death and disability

The charity has a continuing interest in the cardiological applications of magnetic resonance techniques with particular emphasis upon ischaemic heart disease

Applications for the support of research consonant with the charity's aims are now invited Application forms are available from the address below and should be completed for return **no later than 26th January 1990**

Tavistock House North
Tavistock Square,
London, WC1H 9TH
Telephone 01-387 9779

CORDA
the heart charity

(2135)H

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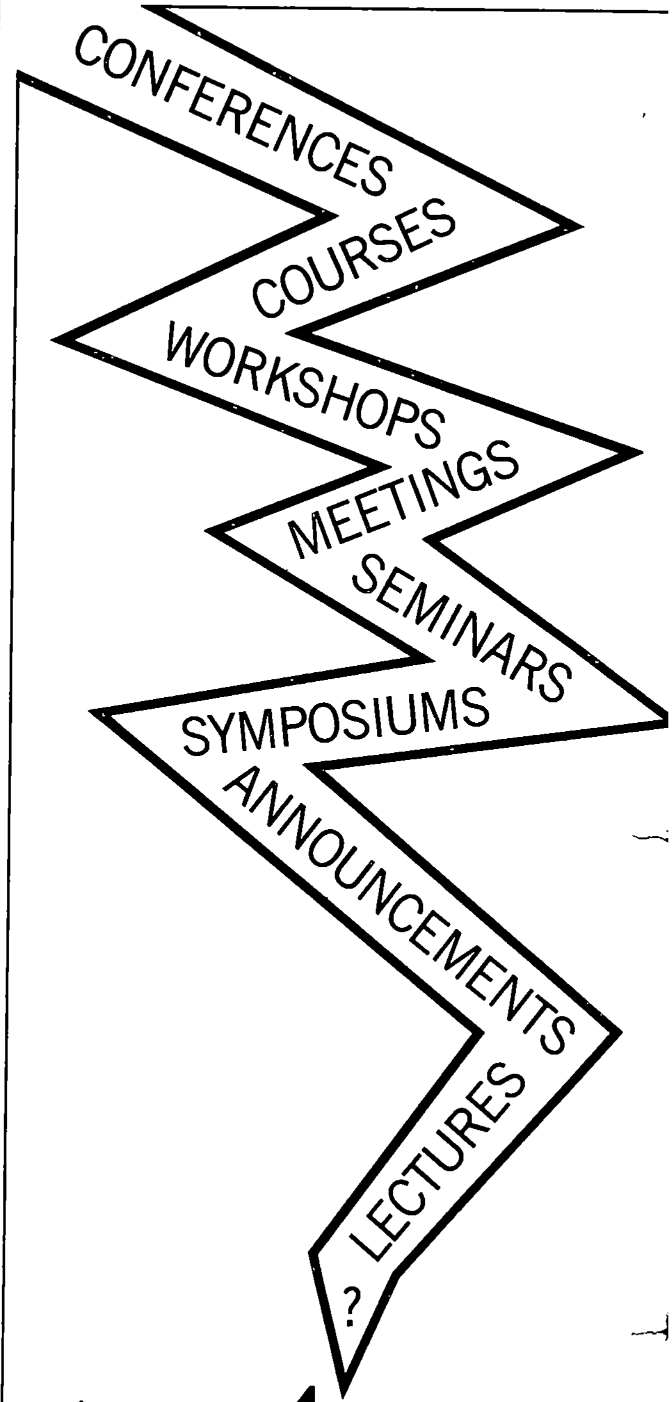

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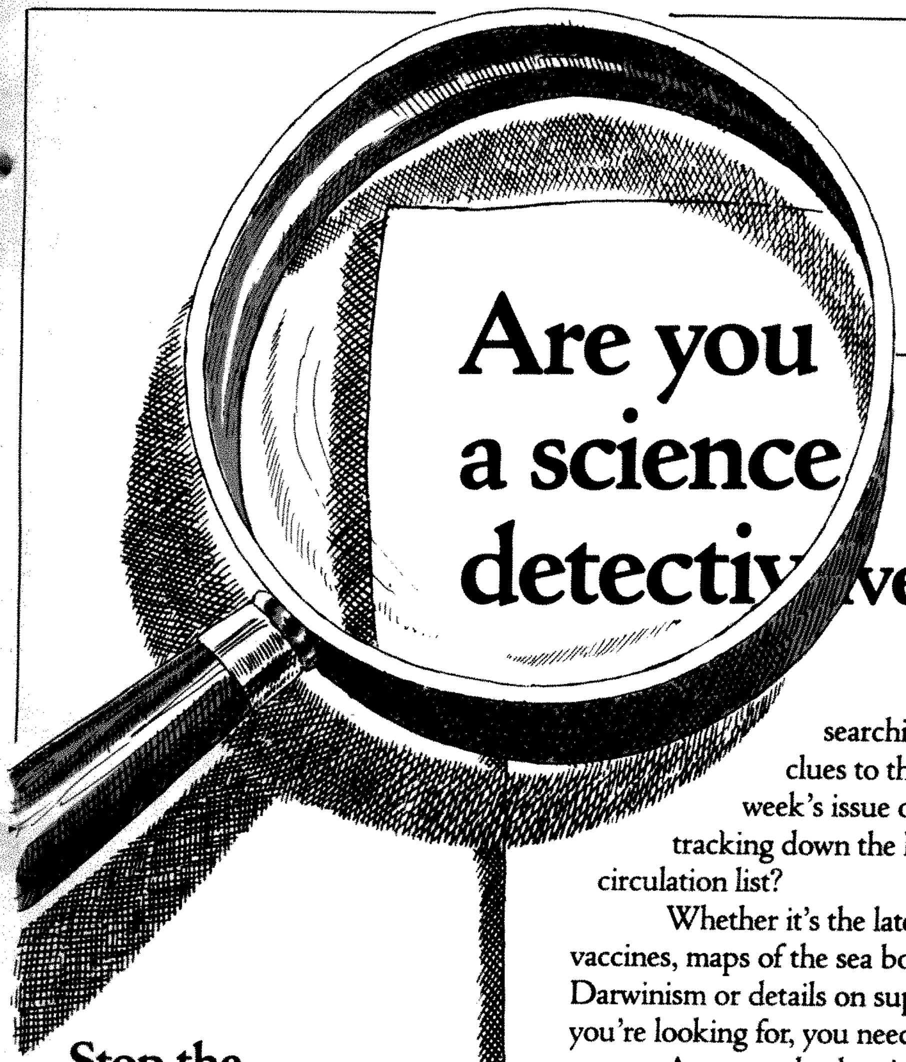

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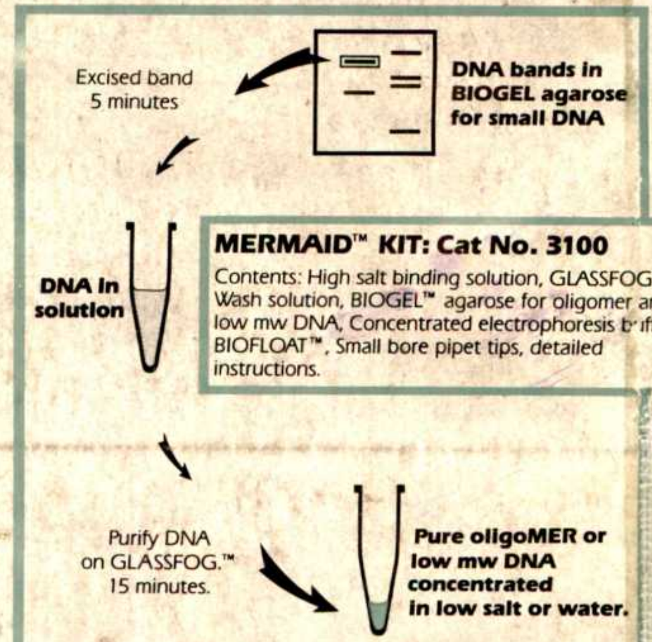

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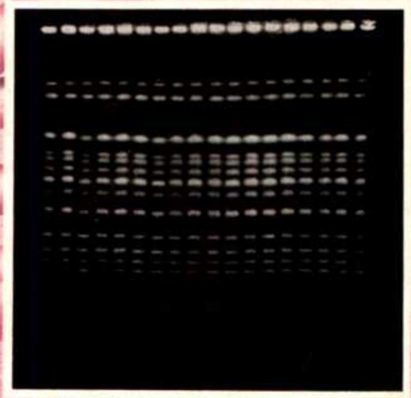

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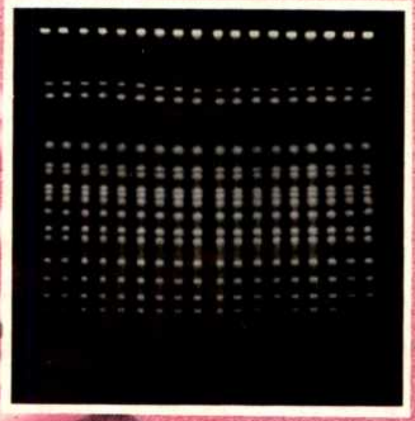

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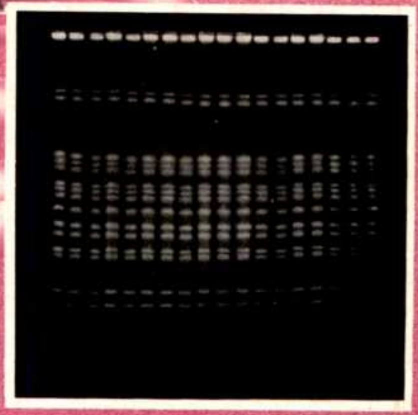

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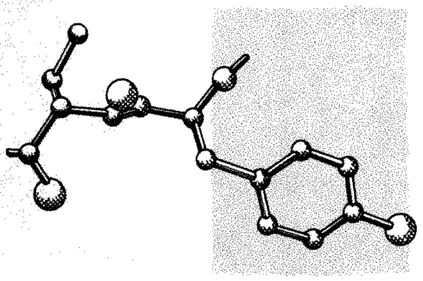

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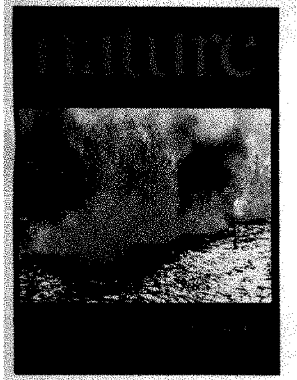

nature

7 December 1989
Vol. 343 Issue no. 6250

◀ Turbulent fountaining of lava above two eruptive fissures near the current site of the vent at Pu'u O'o, Hawaii. Photo by J. Griggs (USGS) in February 1983. A model to account for some of the behaviour patterns displayed by such eruptions is presented on page 665, and discussed in News and Views.

THIS WEEK

Shear rivalry

Rival US and Australian patents on 'gene shear' technology raise the issue of the effect of commercialization on the open discussion of research results, page 609.

Target for HIV

An HIV gene product that plays a part in controlling the activity of the AIDS-inducing virus binds directly to a specific segment of some messenger RNA molecules transcribed from HIV. Page 714.

Glutamate receptor

The expression, cloning and sequencing of a kainate receptor from rat brain provides the first primary structure of a family of receptors believed to play a central role in memory acquisition, page 643. Sequences of kainate binding proteins from frog brain (page 684) and chick cerebellum (page 689) are consistent with their being the binding domain of the functional receptor. See also News and Views page 617.

On the level

A continuous detailed record of sea-level variations over the past 17,000 years has been obtained from studies of coral reefs off Barbados. One of the more surprising findings is that there were two periods of rapid sea-level rise during the last deglaciation. These results give new insight into the relation between ocean circulation and climate change. Pages 637 and 616.

Whipped into shape

Images from X-ray fibre diffraction reveal the arrangement of flagellin subunits within the core of a bacterial flagellum and show that there is a central channel through which the subunits are transported to the distal end of the growing filament. Page 648.

A crucial difference

The 'left-handed' stereoisomer of thalidomide is a powerful and safe tranquilizer; but the 'right-handed' isomer was also present in the drug preparations used in the 1960s, and was responsible for many severe birth defects. An important aim of pharmacology, therefore, is the synthesis of drugs containing only the active enatiomer, and on page 631 Brown and Davies review progress towards chemical synthesis of specific stereoisomers.

Cement relationships

'Cement sickness' due to sulphate attack is afflicting many buildings around the world, but it is often difficult to establish the origin of the sulphate. Sulphur isotope analysis is shown (page 663) to be a useful tool in tracing the source.

RA and development

The three conserved genes for the three types of nuclear retinoic acid receptor are differentially distributed during development of the mouse limb bud, and the timing and pattern of expression of these and the cytoplasmic retinoic acid binding protein suggest how responses to retinoic acid may be regulated during limb morphogenesis. Page 702.

Name dropping

A study of a brain-damaged patient with a selective inability to retrieve proper nouns suggests that these words are represented or accessed in the brain separately as a category distinct from common nouns, page 678.

New old reptile

Fully evolved reptiles existed 340 million years ago — 40 million years earlier than previously thought, page 676.

Guide to authors

Facing page 631.

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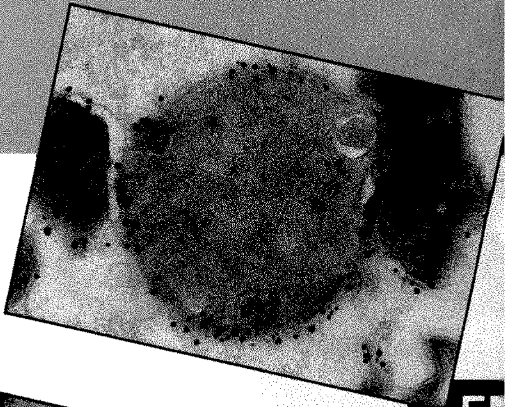
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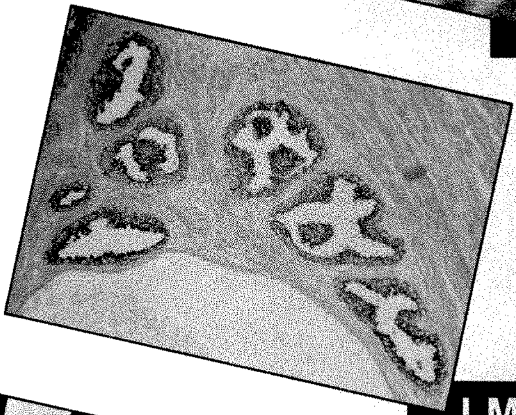
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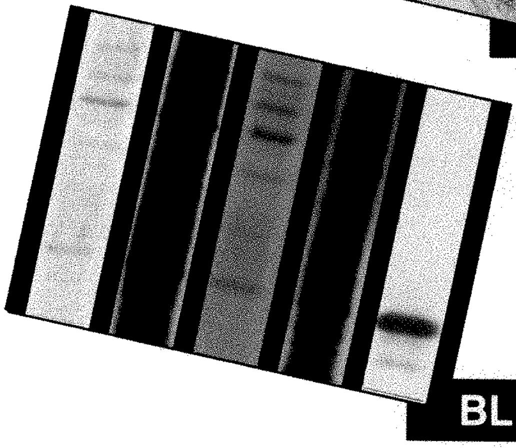
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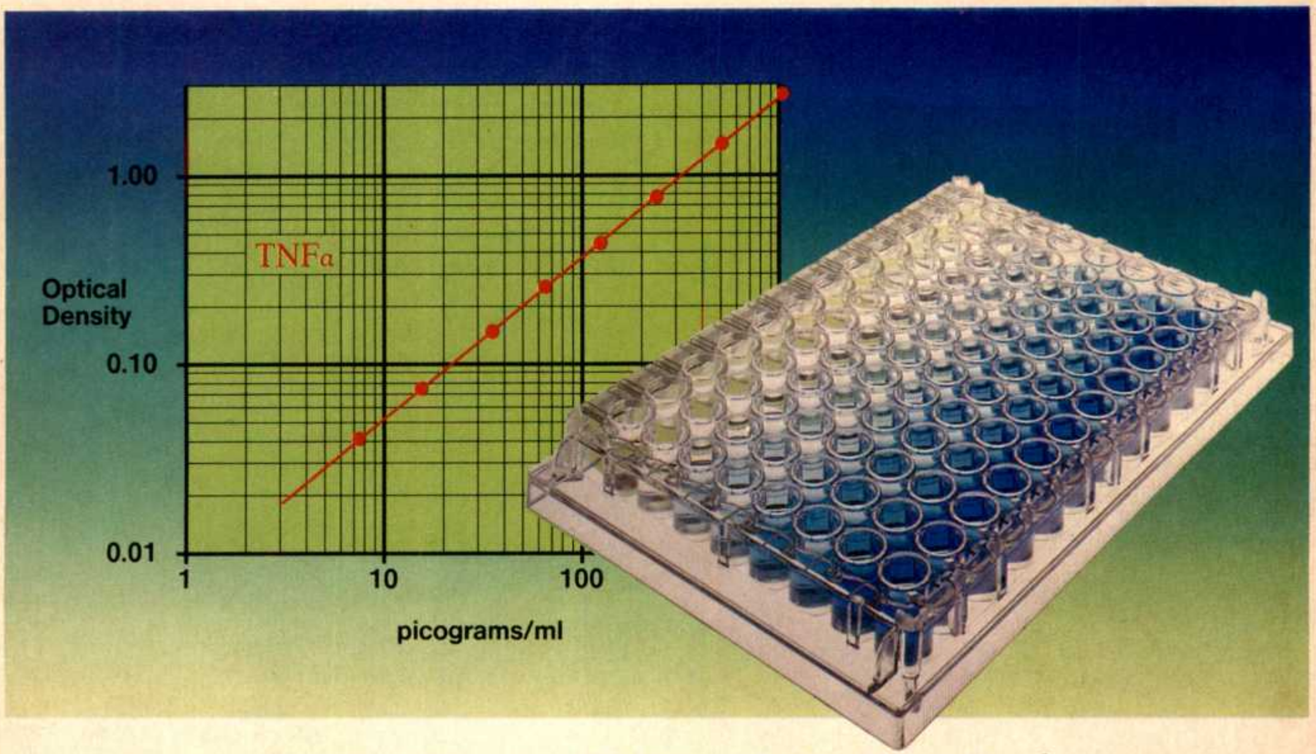

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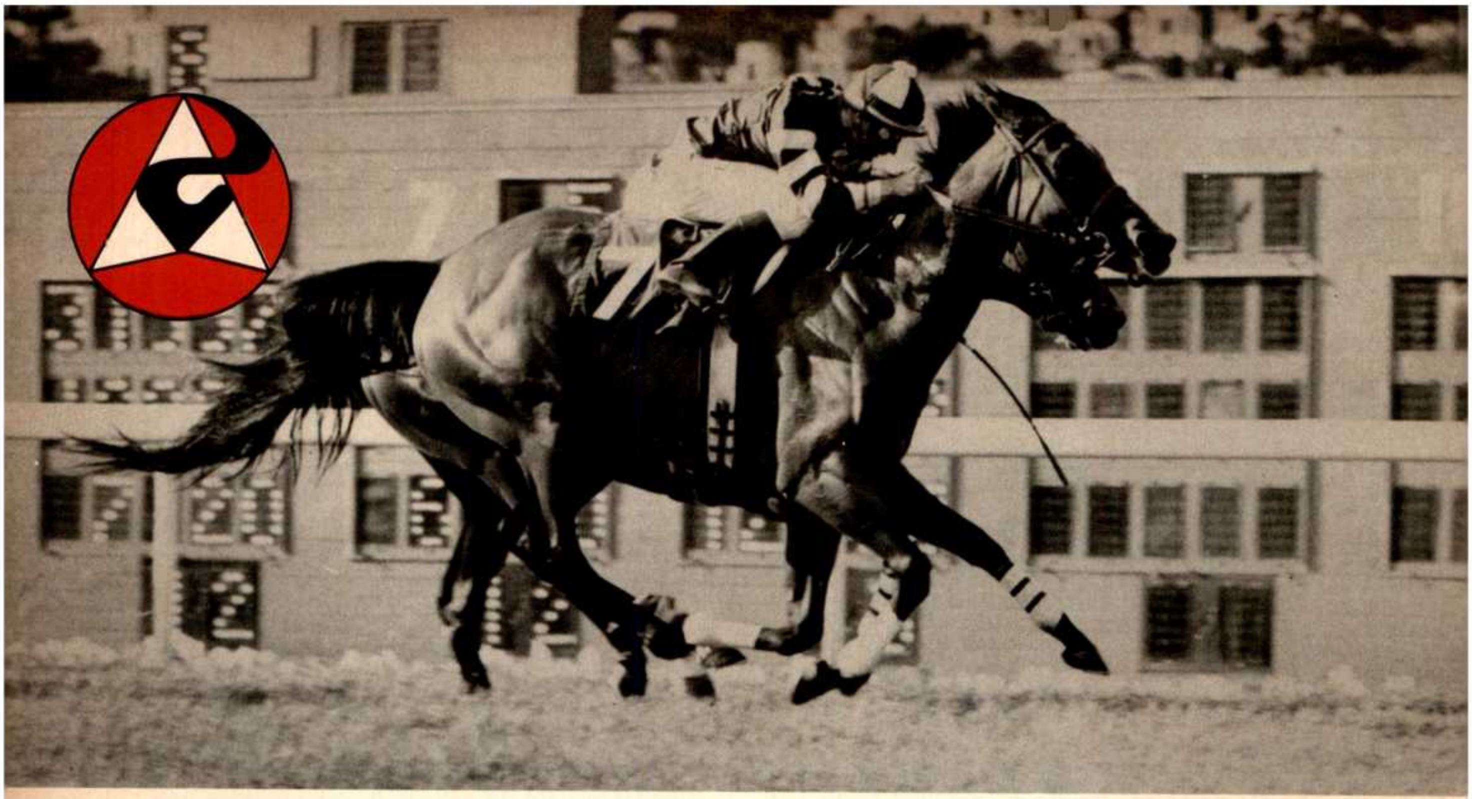

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Antigen Recognition and Lymphocyte Activation
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Molecular Mediators of Immune Pathogenesis
Kendall A. Smith, Dartmouth
Thomas Malek, University of Miami
Marian E. Koshland, UC-Berkeley
Eckhard R. Podack, University of Miami
Anthony Cerami; Rockefeller University

Molecular Pathogenic Mechanisms
MONDAY (afternoon)
Diseases of Abnormal Antigen Receptor Formation
Ilan R. Kirsch, NCI-NMOB
Hitoshi Sakano, UC-Berkeley
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TUESDAY (morning)
Molecular Mechanisms in Autoimmunity
Lawrence Steinman, Stanford
Chella S. David, Mayo Medical School
Bonnie Blomberg, University of Miami

TUESDAY (afternoon)
Disordered Regulation of Lymphocyte Activation
Cox Terhorst, Harvard Medical School
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THURSDAY (morning)
Genetic Disorders of Surface Molecules
Gerald T. Nepom, Virginia Mason Research Center
Bernard F. Mach, University of Geneva
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THURSDAY (afternoon)
Molecular Mechanisms of Immune-Mediated Tissue Injury
Victor Nussenzweig, New York University
Joan Stein-Streilein, University of Miami
Gilla Kaplan, The Rockefeller University

Biotechnology and the Monoclonal Antibody—The Second 15 Years
MONDAY (afternoon)
Receptors, Immunological Structure, and Pharmaceutical Development
Mark I. Greene, University of Pennsylvania
Donald C. Wiley, Harvard
Jeffrey Greve, Molecular Therapeutics
Eckhard Wimmer, SUNY

TUESDAY (morning)
Manipulating the Monoclonal I
James Larrick, Genelabs
Andreas Pluckthun, Max Planck Institute–* Martinsreid
Jorge Gavilondo-Cowley, Center for Genetic Engineering and Biotechnology–Havana

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Manipulating the Monoclonal II
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THURSDAY (morning)
The Biotechnology of Allergy
Kimishige Ishizaka, Johns Hopkins
Kevin W. Moore, DNAX Research Institute
Jean-Pierre Kinet, NIH
Tse-Wen Chang, Tanox Biosystems

THURSDAY (afternoon)
New Directions in Immunogenetics
Ursula Storb, University of Chicago
Tak W. Mak, University of Toronto
Charles J. Epstein, UC-San Francisco

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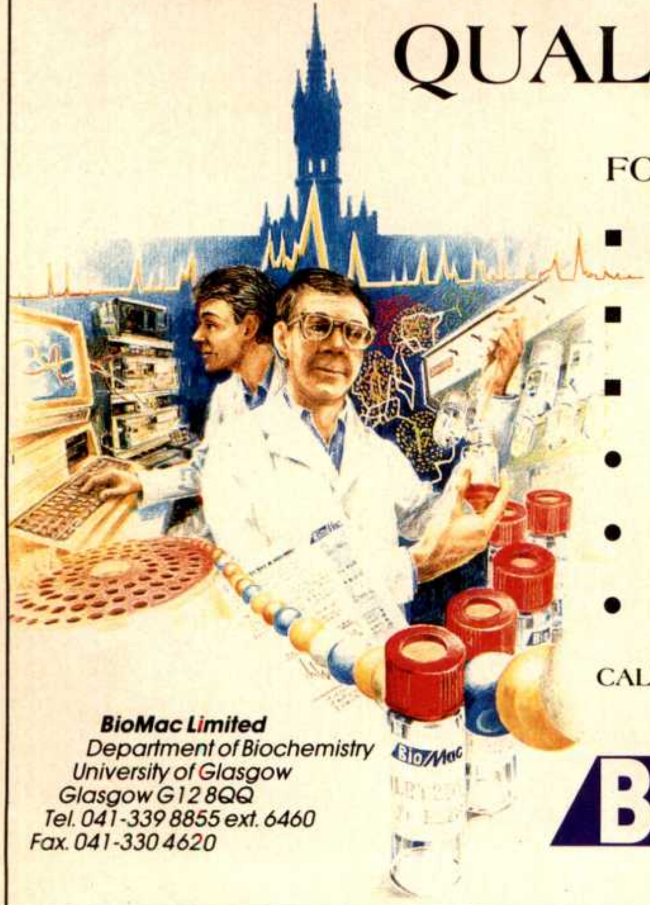

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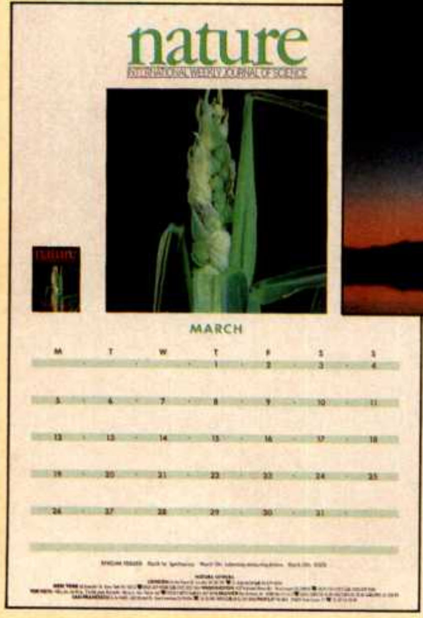

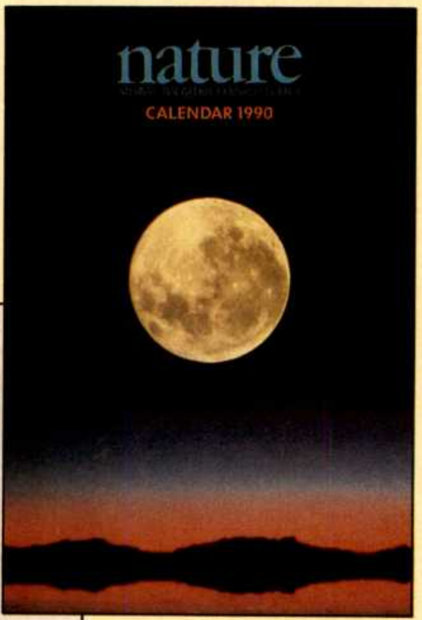

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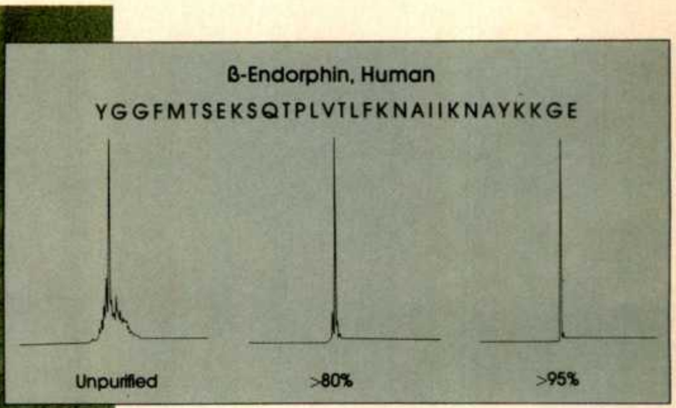

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NATURE VOL 342 nature 7 DECEMBER 1989

Tomorrow's conundrum of Europe

Excitement over developments in Eastern Europe should be moderated by a recognition of the chauvinistic dangers that it brings What Europe needs is a moratorium on boundary changes German reunification is best postponed

MALTA's role in European affairs has not been conspicuous since the ending of the Crusades, while the meeting on (rather than, as originally intended, off) the island between Presidents Mikhail Gorbachev and George Bush will not have restored much of its influence That it allowed the two men to smile often and openly in each other's presence is, of course, a plus, the more serious business on arms control planned for next June should now be the more easily carried through Each, no doubt, will now also be more confident that the other is seriously seeking to divert military spending to civil causes — the US administration seems to have seized more eagerly on he chance to cut spending than other members of the North Atlantic Treaty Organization The federal deficit plainly nags like toothache The Soviet deficit, relatively even larger, is for the time being bridged by printing money, no wonder that Gorbachev is pleased that his new friend, Bush, may use his economic influence (but not his cheque-book) to help *perestroika* along Whether that will help during the long winter is another matter

What seems to have foxed the two presidents at the weekend (the weather apart) is the turmoil in Europe They are not alone in that Monolithic regimes in Eastern Europe have been collapsing like dominoes since the Soviet Union let it be known that it would not prevent political change in Poland, now almost a democracy f an impoverished one But that does not mean that Gorbachev has lost control He seems resolute in telling ancient leaders that they cannot stand in the way of history and equally determined that the East European tates should stay within the Warsaw Pact, German nostalgia notwithstanding The benefits? The expectation hat the conservatives in the Soviet Union will understand hat what goes for Eastern Europe also applies to them But the pace of change has left even Gorbachev wanting or a tangible description of what he means by a "common European home" Bush is as perplexed

It is a perplexing business Three years from now, at the nd (not the beginning) of 1992, the European Communities hope to be a true common market and thus, in due ourse, a dominating economic force in Europe The chiefly French fear that the process will be interrupted by ambitions for the reunification of East and West Germany are not over-serious the frenzy of the past few weeks will soon abate, to be replaced by mutual recognition that reunification is at best a distant prospect Why should two of Europe's three-and-a-third German-speaking countries (counting Switzerland ungenerously as a third) consider they have no choice but to turn the clock back almost half a century, especially when their boundaries have changed considerably since 1939 and when there are much more interesting avenues for them to explore?

The interest of Europe is its diversity So much can be told from comparing a journey from Stockholm to Naples with one from, say, Omaha (Nebraska) to Austin (Texas), roughly the same distance It is remarkable that the countries of Eastern Europe have kept their own distinctive character throughout the past 40 years Now there will be even more diversity to conjure with The danger is that its interest will be complicated by ethnic or, more accurately, linguistic tensions Many of the new near-democracies are artificial states, glued together at Versailles and Yalta, after the First and Second World Wars The temptation now will be to unravel these ancient packages, reuniting patches on the map of Europe as if solving a jigsaw puzzle, sometimes even carrying out gigantic programmes of 'repatriation'

These temptations are natural, but should be suppressed by those in whom they arise In the present heady atmosphere, people may forget that the Second World War began with hankerings of this kind What Europe most needs now is a moratorium on external boundary changes Internal changes are a different matter, if people thrown together in some past jigsaw game so dislike each other, fission should be allowed Nobody would wish the present state of Belgium on, say, Czechoslovakia, but there must be better ways of letting people sense that they are in charge of their own affairs That is why German reunification would be a bad precedent

The better bet for everybody is that mobility, allowed for and even encouraged by the Helsinki agreements of 1979, should have a chance to make all European states more prosperous A precondition would be that the members of the European Communities should be at least as open to other European states as they are to each other That may be easier now that Europe's superpower hankerings have been attenuated with the cold war whose fighting kept them alive Gorbachev's common European home might then be a collection of smaller communities, a kind of Switzerland writ large Maybe that is what he is driving at even in the Soviet Union □

Uranium bites dust

The British government seems bent on letting past investments in nuclear power be wasted

THE British seem to have a flair for indecision from which even the present decision-prone government is not immune The failure to decide what should happen to the goods and people delivered to South-East England by the Channel Tunnel a few years from now would be a joke were it not likely to become a reality Now, a modest programme of nuclear construction has been abandoned after a decade's costly effort by engineers, bureaucrats and lawyers, but for reasons not strictly concerned with the safety of nuclear power or with long-term economics It is as if the British government shares with its predecessors a liking for the sensation of sand running through its fingers

Ten years ago, the government came to office with a plan for building nuclear reactors to the Westinghouse pressurized-water design at the rate of roughly one a year Construction of the first generating station (with two reactors) began two years ago at Sizewell in Suffolk, on the North Sea coast The project has been enormously delayed by the decision that a public planning inquiry should be a 'generic' inquiry, dealing with general matters as well as the suitability of the site The hope was to save time later in the construction programme But the second public inquiry, in respect of a site at Hinckley Point on the Somerset coast (which closed last week) has been almost as long and painstaking Indeed, the inquiry outlasted the nuclear programme itself, which was formally put on ice last month

The explanation is simple, but not straightforward The British nuclear programme has been a casualty of the government's determination to sell the electricity industry to private investors The first stages of this huge exercise will begin next year, with the privatization of the electricity distribution companies The sale of the generating plants, which will follow in 1991, has always seemed more complicated Originally, it was planned that there should be two generating companies (one large and one small called National Power and Powergen respectively) to simulate competition Now there is to be a third, whose plant will consist of all the nuclear stations operating or being built It may just be on the point of going out of business when the greenhouse effect prompts another British nuclear programme

The origins of this development are not obscure, but concern the high costs of operating a series of unsuccessful and uneconomic reactors called AGRs (for "Advanced Gas Cooled") as well as the estimated costs of decommissioning a previous generation of reactors National Power, the larger of the generating companies which was to have had responsibility for nuclear generation, may now kick itself for have lobbied so successfully to shield its future shareholders from hardship by frightening the government with worst-case costs

The effects of the decision are nevertheless curious The new nuclear company will be cast in the mould of the Atomic Energy Authority created in the 1950s as the spear-carrier of the new nuclear industry Over 40 years, the authority has consumed a substantial fraction of Britain's engineering skill, together with substantial amounts of money It remains a technically excellent organization, but without a nuclear programme, it might as well be renamed (its Harwell laboratory has become an excellent engineering and materials laboratory) or even be wound up when the fast reactor programme in Scotland comes to an end Future British governments may find it simpler, on future occasions, just to dig holes in the ground and fill them in again □

Generating physicists

A British minister hopes that compulsory physics will make more physicists, but he could be wrong

STUNG by the example of the Japanese, all industrialized states are these days worrying about the sufficiency of the supply of technical people, scientists and engineers in particular Generally speaking, there are too few of them Generally speaking, the poorer industrialized economies are the most deprived There is no accident in that science education costs time and money, at least in comparison with the language and literature of the region (unless, ironically, the language is Japanese) Even so, it is curious that one of the British government's least celebrated innovations of the past few years is the introduction of a national curriculum for primary and secondary schools, partly with the objective of increasing the supply of scientists and engineers How will it work out?

To judge from what Mr Alan Howarth, a minister at the British Department of Education and Science, was saying the other day, not much has changed Speaking to the Institute of Physics, Howarth said last month that the national curriculum, by introducing students to physics at an early age, must eventually increase the supply But the truth could easily be quite different Past experience in British schools with the old curriculum, allowing for specialization at too early an age, shows that there is nothing like a poor teacher to drive young people into other pursuits Howarth was promising more teachers the other day, but will they be good teachers? And if not, may it not be the worse for science education that young people should be forced to sit through classes that will convince them that they should opt for the arts and humanities?

If Howarth really had the supply of technical people at heart, he would settle instead for a compulsory curriculum that did hardly more than persuade young people that these technical fields are interesting and important, letting them learn to be professionals when they are ready for the task □

Back to the drawing board for Yucca Mountain repository

- DoE admits research was inadequate
- Government to sue Nevada for right to dump?

Boston

PLANS by the US Department of Energy DoE) to build the nation's first and only high-level nuclear waste repository were et back to square one last week when a op-ranking DoE official unexpectedly acknowledged that the department lacks confidence in its research on the facility and will completely revise the project The announcement effectively scraps the $500-million-worth of research on the epository site, at Yucca Mountain in Nevada, that the DoE has conducted over he past two years And the sudden change of heart throws the future of the high-level waste dump into uncertainty, a position that is exacerbated by an imminent legal showdown between the federal government and the state of Nevada

Disclosing the overhaul of the programme at a nuclear industry convention n San Francisco, Deputy DoE Secretary W Henson Moore said that even with 'optimistic assumptions" the new changes will cause a delay of at least seven years in the project As outlined by Moore, the department's revised timetable means that the repository will not open until the year 2010 at the earliest — 12 years beyond the 1998 deadline imposed originally by Congress when it commissioned the high-level waste project in 1982

Further clouding the facility's fate is the fact that the congressional instruction to the DoE is not to build the repository, but merely to assess the suitability of the proposed site, which lies deep in the volcanic rock of Yucca Mountain, 100 miles northwest of Las Vegas, Nevada The new schedule pushes even this approval process beyond the year 2000, and if Yucca Mountain were then deemed unsuitable, the search for a suitable repository site would have to start anew

The DoE's research on the site until now has been roundly criticized by the Nuclear Regulatory Commission and other agencies, as well as by some of the DoE's own scientists Some observers have praised the DoE for its candour in repudiating its previous work The DoE has now promised that future research on the facility will be subjected to review by the National Academy of Sciences, by the state of Nevada and by industry

But news of the programme's setback has been greeted with dismay in the nuclear industry The cooling ponds which between them hold hundreds of thousands of spent fuel rods from the country's 110 nuclear power plants are filling rapidly, many are nearing capacity, and some plants have already been forced to build new temporary waste facilities to allow operations to continue

US electricity utilities have already raised some $3,000 million from ratepayers for the construction of a waste repository More than one sixth of this money has already been spent on the now-abandoned research The DoE, meanwhile, is bound by contracts to begin accepting waste from the nation's electric utilities in 1998, and the utilities, having fulfilled their side of the bargain, balk at the idea of spending more of their money to build temporary storage facilities The DoE is expected to ask Congress for permission to build an interim storage facility in addition to the Nevada site

The state of California has already passed a law prohibiting construction of any new nuclear power plants until the issue of nuclear-waste disposal has been settled Some industry representatives have already begun to step in to this tangle of policy the Electric Power Research Institute, for example, a research group representing the nation's electric utilities, is said to have asked for a role in overseeing the work on the repository

Most observers believe that even the DoE's revised timetable is extremely optimistic Complicating matters further is the resounding rejection of the site by the state of Nevada In two bills passed last spring, state legislators exercised what they believe is their right to veto the Yucca Mountain site But Moore said the Department of Justice will sue the state of Nevada to force it to accept the site

Nevada legislators, including Governor Bob Miller, say they welcome the legal challenge They have opposed the process unflaggingly ever since they were singled out as the only site under consideration by a surprise congressional move in 1987

Most recently, the state has denied the DoE a permit to dig an exploratory shaft at the Yucca Mountain site Robert Loux, Miller's nuclear-waste policy coordinator, says he believes there are at least three separate issues in the case that could ultimately go to the Supreme Court As Loux sees it, the matter hangs on a simple question of state's rights "Shouldn't a state", he asks, "have a fundamental right to preserve its economy and environment in the face of a noxious, unwanted federal project?"

Seth Shulman

HUMAN FRONTIER PROGRAMME

Switzerland knocking on the door

Tokyo

JAPAN's Human Frontiers Science Programme, which opened its European office n Strasbourg on 21 November (see *Nature* 342, 334, 1989), is already attracting the nterest of countries not among the present participants Switzerland has offered support to the tune of $150,000 per year, and Sweden and Australia are also keen to join the programme

Switzerland's possible membership was discussed at a meeting of the Human Frontiers board of trustees in Strasbourg the day before the office opened Although the Human Frontier programme is underwritten by the Japanese government, the board of trustees consists of representatives from the G-7 group of Western nations (Japan, United States, Canada, West Germany, Great Britain, France and Italy) and the European Community (see *Nature* 339, 573, 1989) When Strasbourg was chosen as the European home of the Frontier programme, France made a financial commitment to the effort, and Switzerland has offered to pay for Frontier fellowships and to provide some financial support for grants and workshops In return, the Swiss want a place on the council of scientists that sets research goals for the programme At present, each of the G-7 nations has two scientists on the council

Some of the seven, including Japan, were keen to accept Switzerland's proposal immediately, but representatives of other nations felt that rules for accepting new members should first be established But Toichi Sakata, director of the Frontier office of the Science and Technology Agency, is confident that Switzerland will gain membership after the next trustees' meeting in April Sweden and Australia have also recently made proposals for membership similar to Switzerland's, Sakata says

The meeting included "tough" negotations over the secretariat that will manage the programme in Strasbourg Several nations were "aggressive" in their desire to get secretariat positions, says Sakata But he takes this as a positive sign of interest As well as appointing Sir James Gowans of Great Britain as secretary general (see *Nature* 342, 463, 1989), the meeting also created permanent positions on the secretariat for France, West Germany and Japan Other nations will contribute experts as needed The board of trustees also approved nominees for the council of scientists (European Community members have yet to be nominated)

David Swinbanks

How the earth moved . . .

San Francisco

ALTHOUGH Santa Cruz was the city closest to the epicentre of California's Loma Prieta earthquake on 17 October, the damage was worse in west Oakland and parts of San Francisco, a consequence of the way that complex local geology transmits the earthquake's energy After travelling around the entire Bay Area to survey the degree of ground motion, two teams of researchers from the United States Geological Survey (USGS) in Golden, Colorado, have made a preliminary estimate of the magnitude of the Loma Prieta earthquake according to the Modified Mercalli intensity scale, a qualitative measure of ground shaking, rather than the familiar Richter scale, which measures the energy released at the epicentre

The Mercalli scale runs from I to XII, with magnitude I being imperceptible and magnitude XII corresponding to complete destruction A rating of VIII, which was assessed by the USGS researchers for Santa Cruz and its immediate vicinity, is characterized as the threshold of structural damage to buildings — wood-framed houses move on their foundations and unreinforced masonry walls tumble to the ground But the localities that suffered the greatest destruction, west Oakland (where the Cypress section of Interstate highway 800 collapsed) and the Marina and Market Street areas of San Francisco, were assigned a IX rating

Researchers suspect that thick deposits of alluvium near these heavily damaged areas amplified seismic waves Alluvial layers of unconsolidated sediments and mud ring the perimeter of San Francisco Bay up to 200 feet thick, responding like "a bowl of jelly", absorbing and amplifying the seismic waves passing through the rock beneath, according to USGS geophysicist Paul Thenhaus

As a result, he said, "it's not unusual to get higher ground motions at long distances from the epicentre in a large earthquake" Indeed, similar circumstances beset the 8 1 magnitude (Richter) earthquake on 19 September 1985 that devastated large parts of Mexico City Even though the earthquake's epicentre was 350 km away, the city suffered widespread damage because its central and eastern portions are largely on an old lakebed

The Mercalli rating does not provide a completely clear picture of the Loma Prieta event Single-storey buildings in Santa Cruz fared much worse than those in San Francisco and Oakland, although the latter were shaken harder The discrepancy results from differences in propagation between low- and high-frequency seismic waves, and from varying resonances of different kinds of building (see *Nature* **341**, 676, 1989) Also clouding the analysis was the liquefaction of sandy soils, particularly in the San Francisco Marina district, which led to serious damage even though the ground motion was relatively small in that area To corroborate their assessments, the USGS teams studied areas of similar damage that appeared to be uninfluenced by such ground effects

In addition to their direct observations, the researchers incorporated results from 1,313 questionnaires, distributed to posi masters and police and fire station around California and in parts of wester Nevada and southern Oregon, asking fc eye-witness accounts of the effects of th earthquake Not all the forms, whic include 63 questions with up to seve different answers each, have been re turned Carl Stover, head of the U Earthquakes Project for the USGS, doe not expect a final report, incorporatin data from research papers and othe sources, to be complete for two years

Robert Bude

. . . and the pollution didn't

San Francisco

WHEN the San Francisco Bay Bridge, a section of which collapsed during last month's earthquake, reopened on 17 November after its month-long closure, local air-quality officials at first reported that the shutdown, which forced many commuters to leave their cars at home and travel instead by ferries and trains, had led to a measurable fall in air pollution from automobile emissions

But now officials of the Bay Area Air Quality Management District (BAAQMD) admit they may have spoken too soon An array of complications, including weather and heavier-than-normal car traffic on other bridges, seems to have made doubtful the hoped-for 'case study' of what would happen if people did not use the Bay Bridge

Researchers are now saying that they have no scientific evidence for cleaner air during the bridge closure, a great disappointment to BAAQMD's board of directors, which had hoped to drive home to the public the benefits of staying off the roads

The San Francisco Bay Area, with some 5 5 million residents, is the fourth-largest US metropolitan area Before the earthquake, 243,000 vehicles crossed the San Francisco Bay Bridge daily, and the area's four other main bridges carried a total of 273,000 vehicles These cars, trucks and buses contributed half of the 500 tons of hydrocarbons released daily into the atmosphere, and 90 per cent of the 3,000 tons of carbon monoxide Of the two, hydrocarbons, which are precursors to the formation of ozone and thence of smog, are regarded by air-quality officials as the more serious pollutant

At first, BAAQMD officials simply removed the Bay Bridge vehicles from their calculations, and guessed there should have been a 6 7 per cent reduction in hydrocarbon and carbon monoxide levels while the bridge was closed But figures collected at four alternative bridges showed their traffic went up to 435,000 vehicles a day during the Bay Bridge closure, making up two-thirds o the difference Moreover, the extra traffi caused heavy congestion, and accordin to James Sandberg, section chief o meterology and data analysis for the air quality district, in slow-moving traffic th emission per car goes up

Added to this complication is the fac that ozone forms only when the weather i warm enough to provide the radian energy to drive the needed photochemica reactions And during hot weather a inversion layer of warm air sits over th surface, trapping and concentrating pollutants

In the moderate Bay Area climate ozone levels have never exceeded federa standards from mid-October until earl spring "It's just not our ozone season" said Milton Feldstein, BAAQMD's air pollution control officer

Even if all the cars in the area hac stayed off the roads, there probably woulc not have been a measurable change ir ozone levels

"I hate to say maybe we should have had the earthquake in September when i was nice and warm, because they'll lynch me", said Feldstein

The end result is that ozone level during the Bay Bridge closure were dowr slightly, but did not go beyond the bound of normal seasonal variations, making an useful scientific conclusion impossible But that does not mean the unique case study of the failed bridge is a total loss Researchers are now trying to determine more exactly the number of cars on the road in the month following the earthquake

Taking into account the slower speeds caused by increased congestion, they will then try to make a more accurate projection of emissions reductions and create a computer model to see if the decrease in emissions would have made a difference in ozone season Feldstein said that BAAQMD might then still obtain evidence to convince people of the virtue of staying off the road

Robert Buderl

INTELLECTUAL PROPERTY RIGHTS —

Copyright *vs* patents for software

Washington

Disagreements within the computer science community over the best way to protect software surfaced last week at a discussion meeting organized by the computer science and technology board of the National Academy of Sciences Software has traditionally been protected through copyright laws, but researchers are increasingly taking out patents on their programmes, and are divided over the issue of whether patent protection should be strengthened or weakened

Among the problems created by the recourse to patent law is the delay in processing applications at the US Patent and Trademark Office (PTO), and the ambiguity of some patents issued, which has on occasion erupted into expensive law suits The PTO is also causing irritation among academics and industrialists in the computer science community by issuing patents for software that many consider too unoriginal to merit protection Robert Spinrad of the Xerox Corporation complained that the confusion was having a "stultifying and dulling effect" on the industry Dan Brinklin, now president of Software Garden Inc , who was forced to sell his former company after a successful but expensive patent dispute, said that the software industry is "very scared" by the patent process

The debate centred upon what would best serve the public interest John Schoch of Asset Management Company, a venture capital group based in California, argued strongly that patent protection must be strengthened as this encourages innovation and therefore benefits the public But Anita Jones of the department of computer science of the University of Virginia argued that patent protection should be weakened because the public benefit lies chiefly in the unrestricted dissemination of information

There was also disagreement over the use of copyright to protect software Although some complain about the copyright system, it has the advantage of being enforceable worldwide through international copyright agreements

Howard Figueroa, vice-president of commercial and industry relations at IBM Corporation, was among those who spoke strongly in favour of the current system which relies heavily on the use of copyright His response to heated discussion between those who favour strengthening patent protection and those who would like to see it weakened was "if it ain't broke don't break it"

The 20–25 per cent annual growth in the computer software industry in the past 10 years proves that the current system is working, and the problems could easily be put right he argued

Christine McGourty

INTERNATIONAL PATENTS

Late award reveals problems

Tokyo

After waiting for three decades, the US electronics giant Texas Instruments (TI) has been awarded a Japanese patent for the technology that forms the basis of nearly all integrated circuits The patent is expected to bring in hundreds of millions of dollars in royalties and will provide a powerful bargaining tool for TI in renegotiations of cross-licensing agreements with Japan's electronics companies But although TI has hailed the agreement as a new sign of respect for intellectual property rights in Japan, the prolonged process of patent application only highlights the problems that foreign companies face in protecting their rights in Japan

The newly patented invention goes back to 1958, when Jack Kilby, a TI engineer, showed how to build transistors, capacitors and resistors together on a semiconducting substrate to make an integrated circuit A US patent was applied for in 1959 and awarded a few years later A Japanese patent application was filed on 6 February 1960 But the Japanese patent office objected to the broadness of the application, which was then split into several smaller claims

But still objections were raised and the proceedings delayed, and only on 27 November 1986 was the central part of the original patent application accepted and published by the patent office

Many of Japan's electronics companies immediately filed opposition proceedings but finally, on 30 October this year, the patent was granted

The Nihon Keizai Shimbun, a financial newspaper, reported the patent award two weeks ago and predicted that it could bring TI royalties of as much as ¥80,000 million ($550 million) a year

But most of Japan's semiconductor manufacturers already have cross-licensing agreements with TI, and they are expected to trade other technology rights, rather than pay royalties, when their agreements with TI come up for renewal in the next year or two

But smaller manufacturers, as well as newcomers to the semiconductor trade, which do not have leading-edge technology to barter with, are expected to have to pay out large amounts in royalties, according to analysts

Masahiro Miki, a spokesman for TI Japan, says the patent award shows the importance that Japan is now attaching to intellectual property rights And he points to TI's success in suing Japanese and Korean companies in 1987 for violations of the company's patent rights for dynamic random access memory (DRAM) technology as another good men But he refused to discuss why the Japanese patent office took more than 29 years to process the Kilby patent

Compared to US patents, Japanese patents tend to be much more narrowly defined, each covering minute aspects of an innovation

The Japanese patent office is flooded with about half a million patent applications a year, which take on average 5–7 years to process In the United States, by contrast, 200,000 applications come in every year, and most are dealt with in 18 months

According to Mitsuru Miyata, editor of the newsletter *Nikkei Biotechnology*, the Japanese patent system is designed not for the protection of intellectual property rights but rather for the import and use of foreign technology Japanese companies seldom fight each other over patent rights, he says instead, competitors go out of their way to file narrow patent applications that differ from each other in only some minor respect, allowing them to compete commercially without violating each other's patents

When a foreign company tries to protect a broad technological invention in Japan, it is forced to break down the patent into a series of narrow applications, each of which has to fight its way through the system The delays provide a crucial advantage for Japanese companies If TI had been awarded its patent 5 or 10 years ago, it would have been a far greater financial burden for Japan's electronics giants, because they then lacked the sophisticated technology then they use in cross-licensing agreements with the US company

This is not the first time the Kilby patent has been at the centre of confrontation between the United States and Japan over technology In the 1960s, the Ministry of International Trade and Industry (MITI) rejected initial efforts by TI to set up operations in Japan, according to David Lammers of the Tokyo office of the Electronic Engineering Times But the US company suggested that any Japanese integrated circuit incorporating the Kilby patent would be barred entry to the US market and so MITI backed down, Lammers says

Although TI says the award of its patent is a sign of change for the better in Japan, the irony is that under present Japanese law the patent would never have been granted Under current rules, a patent application expires 20 years after the application is filed or 15 years after publication, whichever comes first, says Yasunori Ohtsuka, a patent lawyer in Tokyo But the TI application in 1960 was submitted just two months before the current law came into effect, and under the old law, which still applies to the US company's case, there is no time limit

David Swinbanks

SOUTH AFRICAN RESEARCH

Science budget falling behind

Cape Town

RESEARCH prospects in South Africa are looking bleak Although next year's budget has yet to receive formal approval, the Council for Scientific and Industrial Research (CSIR) is expecting an increase of at most 4 per cent With inflation running at 15 per cent, and research costs hit further by the weak rand, significant cutbacks are inevitable Even so, science and education seem to be relatively well-treated by the de Klerk government, which is making a concerted effort to curb state spending, a 15 per cent cut in the defence budget, for example, has been suggested

Worst affected has been the CSIR's Foundation for Research Development (FRD), whose responsibility its head, Rhein Arndt, describes as "manpower development in science and technology"

This includes the funding of university research and the provision of student grants Last year, the CSIR's national laboratories were taken from FRD and given to another arm of CSIR, Research, Development and Implementation (RDI) (see *Nature* **332**, 197, 1988) The aim was to move towards 'near-market' research, and the RDI has been successful in supporting research with contracts from government departments and the private sector Last year, 38 8 per cent of the CSIR's income was derived from outside contracts, and this is expected to rise to 41 5 per cent this year In compensation for this move, the FRD expected to get more of the CSIR's overall budget as the RDI became more self-financing But this has not happened this year, FRD was allocated the same proportion of total government support as last, and no change is expected in the coming year The net effect has therefore been a shift of money from basic to applied research

Although the CSIR's grant is negotiated from the Treasury by the Ministry of Trade, Industry and Tourism, the RDI and the FRD budgets are considered to be separate entities, and are dictated by the government The cabinet is currently considering a request by the FRD to be moved to the national education budget, which is in the hands of Minister Gerrit Viljoen He is more enthusiastic about basic research, and also seems to have the ear of President F W de Klerk

The FRD also runs the country's four national research facilities the South African Astronomical Observatory, the Radio Astronomy Observatory, the Magnetic Observatory and the National Accelerator Centre (NAC) Last year, NAC alone cost CSIR R30 million ($12 million), 34 per cent of the CSIR budget, and many of the country's scientists feel that NAC is an under-used and unaffordable luxury

Last month, FRD initiated an independent review, by scientists from outside South Africa of the quality of work being carried out at NAC, its impact on the development of technical expertise in South Africa, and NAC's place in the country's science system Arndt says that the future of NAC is "something that the science community will have to take a hard decision on"

At the root of all these problems is the small proportion of gross domestic product (GDP) that South Africa spends on research and development In 1987, research and development spending from all sources amounted to 1 per cent of GDP (R1,700 million, or $700 million), with roughly equal amounts coming from government and the private sector This is a much smaller proportion than is spent by Britain or France (2 3 per cent of GDP in 1987), and is less even than India (1 1 per cent) **Michael Cherry**

SPACE AGENCY

Arianespace forced to twiddle thumbs

Paris

TECHNICAL problems, small print and the San Francisco earthquake have left Arianespace, Europe's commercial satellite launch consortium, with nothing to launch this month, despite a full order book and a tight schedule

Originally, the French SPOT 2 commercial Earth observation satellite — the overdue replacement for the successful but ageing SPOT 1 — was scheduled for launch last month But 'technical problems' with the Ariane 40 rocket led Arianespace to delay the Flight 35 launch until December, pending further tests

However, the Superbird A satellite, built by Ford Aerospace for the Japanese, was also scheduled for a December launch and a postponement would have incurred penalties for Arianespace

So the consortium asked the French national space research centre (CNES), which owns SPOT, to let the Japanese satellite go up first

Because Spot 1 is still sending pictures, Spot 2 was rescheduled for launch on 10 January. In the meantime, Superbird A, which was undergoing final tests at Ford Aerospace in Palo Alto, was shaken by the San Francisco earthquake, and the manufacturers decided it would be unwise to send the satellite to the Kourou launch site without more tests As Spot 2 could not be taken out of wraps again at short notice, this has left Ariane-space with two launchers ready to go, but no payload

Peter Coles

SCIENCE EDUCATION

Technical studies to be compulsory

Sydney

AN ambitious reform of the education system in New South Wales will make the study of mathematics, science and technology compulsory for high school students in the province Under the present system, the only compulsory subject for final year students taking their Higher School Certificate is English, but by 1992, following the introduction of the government's white paper (policy document) on curriculum reform, students will also have to take at least one technical course

Until now teachers have been providing courses in technology and computing, of a mostly vocational nature, under a category known as Other Approved Studies (OAS) The government intends to reduce the OAS from 10,000 to 500 subjects, substituting new technology courses instead The white paper will also make it compulsory for 13–15 year-olds to study a language and a technology course In other changes to the curriculum, courses involving AIDS and drug education will be integrated into a new mandatory health and physical education programme

The government acknowledges that the major difficulty in the introduction of these reforms is a shortage of teachers of technology, science and mathematics A spokesperson for the Minister for Education said that the department would be negotiating with the NSW Teachers' Federation for a salary increase for staff in these fields But the teachers are unlikely to go along with such changes Sue Simpson, spokesperson for the NSW Teachers' Federation, said she found the suggestion of differential rates of pay surprising, and argued instead for "an overall increase in teachers' salaries to entice teachers back into the system"

Tania Ewing

ASTRONOMY FROM SPACE

Solar max falls at last

Washington

THE Solar Maximum Mission satellite (SMM), launched in 1980 to observe flares and other activity during the peak of the last solar cycle, almost survived until the peak of the present solar cycle, expected in about four months But last Sunday morning, 3 December, it re-entered the Earth's atmosphere and burned up over the Indian Ocean near Sri Lanka In 1984, SMM became the first satellite to be successfully retrieved, repaired and returned to orbit by the crew of a space shuttle mission SMM's swansong was its observation of gamma-rays from Supernova 1987A, which provided the first observational confirmation that supernovae remnants are powered for the first year or so by the radioactive decay of ^{56}Ni synthesized in the initial explosion (see *Nature* 331, 416, 1989) David Lindley

EAST GERMAN GREENS

Grappling with dirty past

Munich

THE East German environmental movement took its first shaky steps toward political responsibility last week with the formation of the 'Green Party of the German Democratic Republic' In its first statement, the party criticized materialism and advocated reducing environmental pollution

But the party founded over the objections of other ecologically minded people in East Germany, will have to develop a more solid programme in the next few months if it is to achieve even a fraction of its goal of ecological reform in a socialist framework Nevertheless, the founding of the party was considered significant enough to be reported widely in the East German media and to attract support from the West German Green Party

Co-founder Gerhard Bacher admits that winning support will be difficult in a country where everyone pays lip service to ecology Even members of the ruling Communist Party have called for an "ecological reconstruction" of the country Bacher says that the Greens have received anonymous phone calls from Communist Party members insinuating that they have shown right-wing tendencies

A bigger threat is the fragmentation of green thought between the new party and other left-wing groups such as New Forum Bacher claims that the difficulties have been dealt with, but a West German Green who visited last week said that many disagreements remain Members of the opposition movement New Forum also fear that they could be split by difficult choices between preserving the environment in East Germany or opting for profitable but polluting activities that bring in hard currency The environmental side had been gaining ground in East Berlin earlier this year, but the collapse of the government and the need to earn Western money may turn the tide

If it can arrive at a common platform, the Green Party will have a lot to do in East Germany, which has always relied for energy on soft bituminous coal Huge strip-mining machines have churned up the countryside, and when the coal is burned, sulphur compounds are released directly into the atmosphere Smog is a permanent problem in all East German cities, made worse by the black, foul-smelling exhaust from the two-stroke engines of Trabants, the least expensive East German cars

The East German Greens are probably the only Green Party in the world that favours nuclear energy, but they support it only for want of alternatives But their support does not extend beyond completing the nuclear power plant at Stendahl that is under construction They are against new plants, but can suggest no likely alternatives that East Germany can afford

Although East Germany has never had a significant nuclear accident, the uranium mines in the south of the country have been a continual health threat for East Germans who live nearby The East German government has begun to publish radiation and air pollution figures for the first time in its history

The East German Greens will be preoccupied with such basic issues for a long time before they need to consider genetic engineering and other technologies that exercise the West German Greens

What may be the biggest challenge fo the Greens in East Germany has little t do with ecology Bacher warned agains a "disturbing" tendency to favour reunification with West Germany as a way t help East Germany out of economi decay, saying that the West German styl of economic success has its consequences not least for the environment "People' eyes popped out" on their trips to th West during the first weeks after th opening of the wall, said Bacher, but h and his party hope to convince them tha they should consider the price befor buying the goods

Steven Dickma

IN VITRO FERTILISATION

Reformation of advisory board urged

Washington

THE US Congress can now be added to the list of agencies trying to prod the US federal government into reconstituting the Ethics Advisory Board (EAB), the committee with responsibility for overseeing research on *in vitro* fertilization (IVF) In a report issued this week, the House of Representatives committee on government operations urges the government to abide by the regulations of the Department of Health and Human services which require it to set up the board It is an "embarrassment" to the department and to the Public Health Service that the last five secretaries for health have ignored these regulations, says the committee

It also urges the present Secretary for Health, Louis Sullivan, to implement the board's recommendation, made in 1979 before its charter was allowed to expire, to exempt research on embryos less than 14 days old from the need to be reviewed by the EAB Other restrictions would be adequate, such as requiring the informed consent of the couple from whom the embryo was generated or restricting the number of eggs that can be fertilized

Reinforcing the comments made by the Institute of Medicine in a report issued in November (see *Nature* 342, 218, 1989), the committee complains of the inadequacy of current IVF research and the poor infertility services available

In line with the controversy surrounding this issue, five of the 15 Republican members of the committee signed a dissenting statement which says that there is no evidence that significant increases in federal funding for this research will improve its quality It also says that there are fears that an increase in funds for infertility research could strip funds from AIDS research, and that, because the private sector is involved in this research, this is "not the best use of scarce tax dollars"

Christine McGourty

COLD FUSION

An old-fashioned love-song

Tokyo

Cold fusion reared its head again las week in Japan with two independen research groups claiming to have detecte strong neutron bursts from electrochemical experiments The news was splashe across the front pages of newspapers reported on national television an picked up by the US *Wall Street Journal* But Japanese experts on neutron monitoring are sceptical

Kunihide Nishizawa and Nobuhik Wada of Nagoya University say the detected neutron emissions 20,000 time background levels from palladium electrodes that had been soaked in deuterium gas then subjected to a high voltage Clos on the heels of this announcement, a grou at Osaka University, headed by Yoshiak Arata, claimed to have detected neutro emissions 25,000 to 2 5 million time background levels when a voltage wa applied to thick palladium rods immerse in heavy water

But, according to one Japanese expert the detector used by both groups, called a BF3 counter, is intrinsically very sensitive to vibration, and sometimes shows pulse like signals unrelated to neutron emission Wada and Nishizawa should presen pulse-height data before they clain neutron detection, he says

The Osaka University results are more highly regarded, because multiple detectors were used and pulse-height data were presented But neither group has examined the energy spectra of the neutrons and thus cannot claim that fusion is the cause, experts say As one researcher from Japan's new National Institute for Fusion Science put it "we want evidence that the signals arise from neutrons due to nuclear fusion processes" but "some Japanese scientists still sing an old-fashioned love song composed by Pons and Fleischmann" **David Swinbanks**

SPACE EXPLORATION

NASA outlines path to Mars

Washington

PRESIDENT George Bush's vision of lunar and martian exploration, made public on the twentieth anniversary of Apollo 11's Moon landing (see *Nature* **340**, 249, 1989), is so far no more than a vision Trying to inject some reality into these grand schemes, the National Aeronautics and Space Administration (NASA) last month released a report that offers five exploration strategies

But the report, the result of a three-month collaboration by all of NASA's divisions, confines itself to making suggestions, which come without price tags, that the president's National Space Council might wish to adopt with a view to working them up into detailed plans

In the first strategy, the astronauts return to the Moon in 2001 and occupy a lunar base permanently in the following year, the first mission to Mars is in 2015 But this tight schedule depends on accelerating the development of the space station so that its assembly is complete by 1997, two years earlier than planned

Financial problems have already forced NASA to plan a new 'rephased' space station programme which stretches out several parts of the programme by six months The Shuttle-C, a new cargo-carrying launch vehicle needed to ferry space station components from Earth, is the only new vehicle that could be ready in time to meet this schedule

In the second strategy, the first lunar mission is also planned for 2001, but the first human Mars landing is brought forward to 2011, delaying scientific research on the Moon but accelerating it on Mars This would require the concentration of funds in the first decade of the next century so that hardware for both the lunar and the martian outposts could be developed in parallel

NASA also outlines a third possible plan which is similar to this one, and a fourth which delays the major milestones by two to three years

In the final strategy, all the major steps in the programme are delayed the return to the Moon is in 2004, the first landing on Mars is in 2016 and a permanent Mars outpost would not be ready until 2027 Research on the Moon would begin around 2007 with Earth-controlled geological and geophysical exploration, establishment of the astronomical telescope arrays and human biomedical research in the lunar laboratory In 2022, there would be a piloted flight to the far side of the Moon for geological exploration and to set up a radioastronomy and geophysical observatory

To carry out this programme, NASA says it will need a substantial increase in staff numbers, from the 24,000 permanent staff it has at present But it complains in the report about the "antiquated" federal employment structure which keeps salaries and benefits low, making it difficult to recruit high-quality scientific and technical personnel in the increasingly competitive marketplace

NASA also wants to change the rigid budget process which allocates funds on a two-year basis, resulting in short-term planning and instability When NASA was established it was granted "no year" funds which it did not have to spend within an established time period, and it had greater flexibility to alter programmes, to accept gifts, to buy property and to enter into contract on any terms and conditions it deemed necessary With new legislation and regulations, these powers have been gradually eroded, but NASA says that the complexity of a 30-year lunar and Mars exploration programme necessitates change NASA has not yet attempted a programme of this size, requiring such long-term development, and unless existing legal regulatory structures are changed, there will be "a severe penalty in schedules, resources and efficiencies" it says

Christine McGourty

MOLECULAR BIOLOGY

Belgium joins EMBL

Heidelberg

BELGIUM has become the fifteenth member of the European Molecular Biology Laboratory (EMBL), director Lennart Philipson announced last week The additional support of roughly DM1 5 million ($840,000) a year, plus an entry fee, is a modest addition to EMBL's budget, but the new funds, says Philipson, will allow EMBL to increase the number of fellowships it awards The exact use of the money must be determined by the EMBL council Like all member states, Belgium will contribute to EMBL in proportion to its gross domestic product Its yearly payment will provide about 3 1 per cent of the DM49 million ($28 million) EMBL budget

Steven Dickman

CONSERVATION

Too many cooks preserve diversity

Washington

THE preservation of biological diversity in the United States and worldwide is a project with many supporters Paradoxically, that seems to be causing trouble Earlier this year, the National Science Foundation (NSF) argued that, because of its long-standing support of ecological research, it should be given a role in national efforts to document and preserve plant and animal species (see *Nature* 340, 585, 1989) Meanwhile, plans have been stirring in Congress to set up a National Center for Biological Diversity with the Smithsonian Institution at its helm, but at a congressional hearing last month Robert Jenkins, vice-president for science programmes at the Nature Conservancy argued that his organization ought to play the lead role because of its past and continuing efforts in species preservation

The purpose of the hearing, before a subcommittee of the House of Representatives committee on Merchant Marine and Fisheries, was to discuss two principal questions embodied in proposed legislation the establishment of a national centre to coordinate data collection and research concerning biodiversity, and to incorporate into the charter of various federal agencies language to make species preservation a particular concern At present, only the National Forest Service (NFS) has such language in its charter David Wilcove, an ecologist with the Wilderness Society, argued that the NFS had not traditionally put conservation issues high on its list of priorities, and cited the case of the northern spotted owl, which inhabits forests of the Pacific northwest NFS forest management, Wilcove claimed, had given over such quantities of the owl's habitat to logging that the Fish and Wildlife Service, another federal agency, was now arguing for the owl to be listed as an endangered species

It was agreed by all that the Endangered Species Act was ineffectual in preserving general biodiversity, and that some mechanism was needed to enforce conservation regulations before species were threatened to the point of extinction But while there was general satisfaction at the hearing with the idea of a national centre run by the Smithsonian, Jenkins protested that the Nature Conservancy, a private non-profit organization, had been running such a programme on a small scale for many years

Its Heritage Program, he said, was ready to receive federal support that would expand it into the National Center for Biological Diversity of proposed legislation, and suggested an amendment of the law that would give the Nature Conservancy the appropriate leading role But Jenkins seemed to be in a minority of one George Davis of the Academy of Natural Sciences of Philadelphia said there was "plenty the Nature Conservancy is not doing" and that only a suitable federal body would have the authority to enforce interagency collaboration

David Lindley

Correction

The outgoing president of the South African Council for Scientific and Industrial Research is Dr Chris Garbers (see *Nature* **342**, 334, 1989) □

Fair shares for Japanese

SIR—Japan's government institutions are now advertising their scheme for hundreds of foreign postdoctoral fellows (see S S Siddiqui, *Nature* **340**, 337, 1989 and recent advertisements in *Nature* Classified) It is good to know that our research institutions, as well as universities, have finally decided to open their doors to researchers from outside Japan I welcome our government's decision to make its laboratories international, its efforts (including high payment) will be appreciated by foreign researchers and probably by politicians, in spite of the difficulties experienced by foreigners living in Japan

At the same time, however, it is regrettable that non-tenure-track Japanese researchers cannot similarly join laboratories as fellows The time is now ripe for our laboratories and universities (both now training too few post-PhD fellows) to show Japanese PhDs how they can carry out postdoctoral research in their own country If it does not do so, Japan will miss an opportunity to keep promising young scientists wanting experience in laboratories in different fields

YASUSHI SAOTOME

M D Research Co Ltd,
760 Morooka, Kohuku,
Yokohama 222,
Japan

SIR—I read with interest the article by S Berliner III (*Nature* **341**, 379, 1989) pointing out the difficulties of using the 2,000 characters of the Japanese language As he recommended, the Japanese should, as a short-term measure, use bilingual signs in Japanese and English in public places, and should transcribe Japanese characters into roman letters for foreigners As a long-term solution, however, do we really have to reform our language, which has the merits of both ideograms and phonetic signs? During my five years in New York, I found that English-speaking people overlook the fact that the language–brain interface is more important than the language–machine interface

If a sentence written with simple characters like the alphabet is easy to understand, why is machine language composed of only 0 and 1 so difficult for humans? The brain is well suited to recognizing patterns such as faces (*Brain Res* **342**, 91, 1985) and ideograms, and a concept is easily associated with the corresponding ideograms even in a patient with aphasia (*Cortex* **8**, 265, 1972) Japanese sentences are easy to read because they are composed of lexical portions, written with *katakanas* (phonetic signs to express foreign words) and 1,945 basic Chinese characters, and grammatical formatives with *hiraganas* (the other phonetic signs)

In fact, the circulation of newspapers in Japan is the highest (58 per cent) in the world (20 per cent in the United States), and many attempts to persuade Japanese to read newspapers and textbooks written in English or roman-lettered Japanese have failed The percentage of total time spent on language during elementary school is only 25 per cent in Japan but over 40 per cent in the United States, and there is almost no illiteracy in Japan This easy Japanese education has resulted in a flourishing society

The disadvantage of Japanese in writing has now been overcome by the widespread use of Japanese word processors and facsimile machines Memorizing ideograms is easy for children, but not for adults Thus, millions of biological species and chemical compounds must be written in *katakana* instead of 80,000 complicated Chinese characters (*Biochem Educ* **16**, 37, 1988)

People should choose a language suitable for both their brain and their culture, not for the typewriter

YASUO KAGAWA

Department of Biochemistry,
Jichi Medical School,
Tochigi, 329-04, Japan

Don't blame us

SIR—Your leading article "Research by numbers" (*Nature* 341, 674, 1989) quotes from a document setting out new resource management arrangements agreed last April between the Department of Education and Science (DES), the Treasury and the research councils, and wrongly suggests that these arrangements provided the basis for the government's decision not to finance the proposed national survey of sexual attitudes and behaviour

Your readers will want to know that under these arrangements it is for the research councils to decide whether to refer matters to the DES The initiative in seeking ministerial guidance is placed with the research councils, not with ministers

In the case of this survey, the government decided it would not be appropriate to provide financial support from the Department of Health's research programme or, more generally, to sponsor the survey The Economic and Social Research Council (ESRC) decided not to proceed on this basis Contrary to your account, the ESRC was not required to refer the matter to the DES nor did it do so

The resource management agreement was concluded after extensive discussion between the department and all the research councils It is designed to give greater freedom to councils in exchange for departmental scrutiny of councils' management systems It is not the purpose of the new arrangements to limit councils' freedom nor to intervene directly in their scientific judgements Any such agreement would have been unacceptable to the councils which continue to operate under the well established 'arms-length' relationship with the government, embodied in the 1965 act establishing the research councils

ROBERT JACKSON
(Parliamentary Under-
Secretary of State)

Department of Education and Science,
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York Road, London SE1 7PH, UK

Creative force?

SIR—If A Travis (*Nature* **341**, 10, 1989) is personally having "flirtations with the untestable" and holds the belief that there is a "creative force manifesting itself in the observable universe", how does telling us this assist in furthering our understanding of nature? Surely you have received correspondence from others on more important and specific topics that better deserve column space?

The essential difference between religious beliefs such as Travis's and the "organized common sense" of science is that religion demands explanations, while science is, or should be, prepared to answer Nobody knows", and continue with its investigations

Formulating unanswerable questions and then demanding answers to them is a waste of time Formulating a theory to explain a feature of nature and then finding ways to test the theory is not If a way is not found to test the theory, then it must be shelved until one is A theory that life evolved here on Earth out of molecular interactions is in principle testable for example, we can perform individual steps in the process experimentally, we can simulate it in our computers and we may one day be able actually to do the whole thing ourselves The theory may be proved wrong, so therefore it is testable No one has come up with a testable alternative yet, so if this one is proved wrong, we must return to "Nobody knows"

Travis looks for and finds some "unknown creative force" in this theory That can only stem from his own religious desires for there to be one, for there is no mention of one in any of the statements of the theory that I have read

To me, the conclusion "Nobody knows" is far more awe-inspiring and stimulating than "we can't explain this, so therefore there is a mysterious creative force in the universe", which in my opinion simply shows a lack of imagination

K P APPLEBY

35 Catherine Street,
Cambridge CB1 3AW, UK

Q: Why do we have radioactive waste anyway?

A: *All industries produce waste The nuclear industry is no different If we want the benefits of nuclear electricity and nuclear medicine we have to accept the waste But even if we could stop using nuclear technology, waste that already exists would still have to be safely disposed of*

Q What sort of waste is UK Nirex Ltd responsible for?

A *Only low and medium level radioactive waste Low level waste has been routinely disposed of for 30 years and medium level waste has been stored*

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Q: Why isn't radioactive waste stored in the place it's produced?

A: *Many reasons In particular it will be less safe in the long term to have stores dotted around the country close to man and the environment than to have one high quality disposal site protected by hundreds of feet of rock*

Q· What is the likelihood of radiation escaping and harming people?

A: *The chances of anyone being harmed are less than one in a million, which is about the same as the risk involved in smoking just one cigarette in a year Nothing can "leak" in the normal sense, as all the waste in a disposal centre will be set in concrete*

Q· What's to stop radioactivity leaking into the environment?

A *Multiple barriers are placed between the waste and the environment These include steel drums, layers of concrete and the carefully chosen surrounding rocks, which will contain the waste for many thousands of years*

Q: How will wildlife and plants be affected near the site?

A *Nirex will take great care to make sure that the environment is safe and the site will be carefully surveyed to protect any rare or unusual species*

Q Who is responsible for making sure the highest safety standards are upheld?

A· *Ultimate responsibility lies with the Secretary of State for the Environment along with the Secretaries of State for Scotland and Wales UK Nirex is also monitored by the Department of the Environment, the Ministry of Agriculture Fisheries and Food and the Nuclear Installations Inspectorate, as well as receiving advice on procedures from the National Radiological Protection Board*

UK Nirex Ltd , a company owned by the Central Electricity Generating Board, British Nuclear Fuels plc, the UK Atomic Energy Authority and the South of Scotland Electricity Board, is to develop and build a deep underground disposal centre for radioactive waste

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SAFE FOR THE FUTURE

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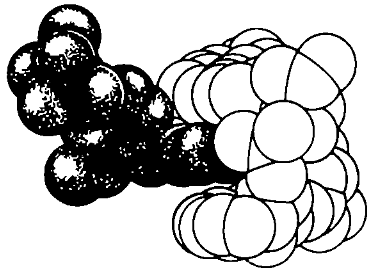

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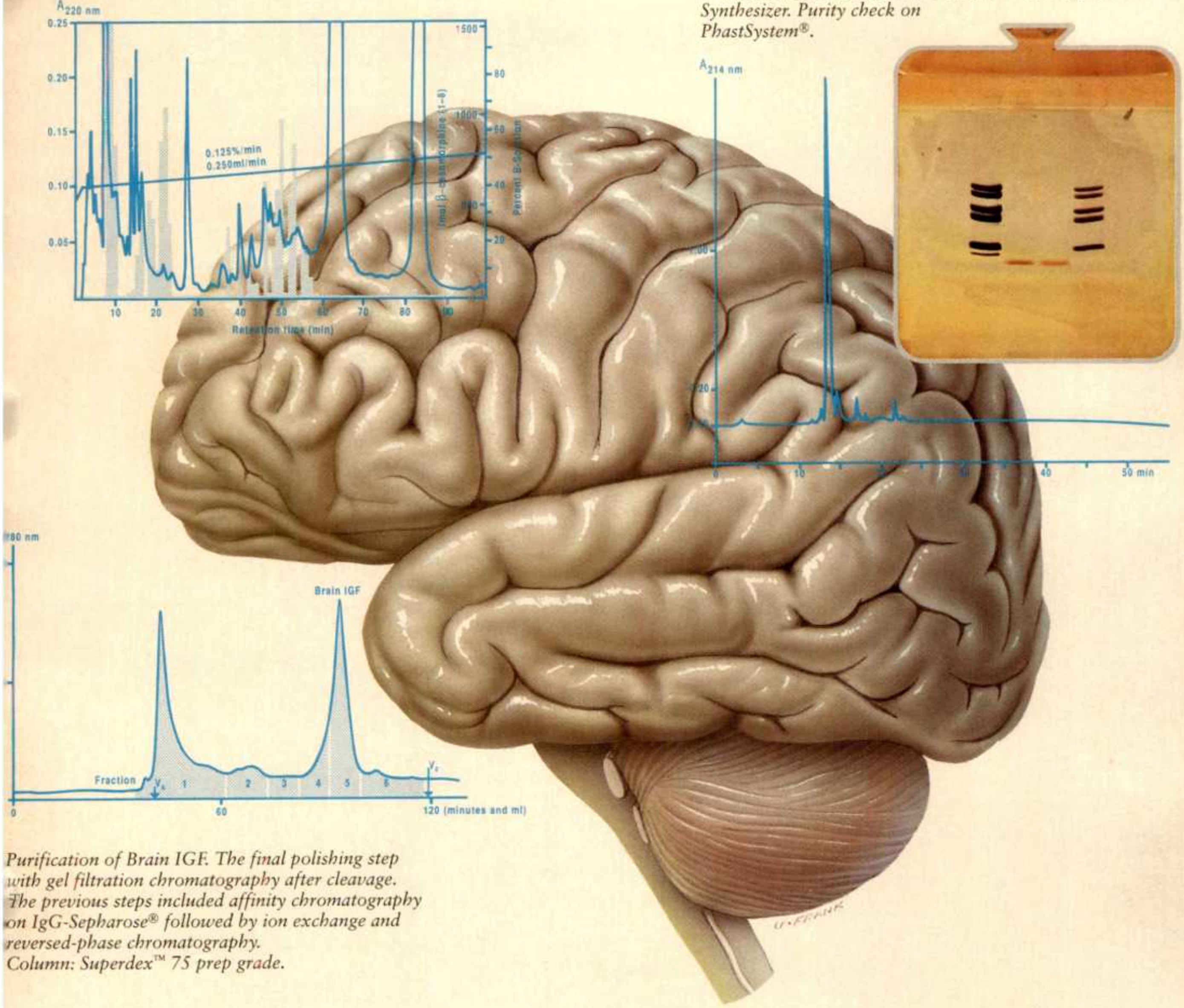

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The great gene shears story

John Maddox

Nature has been in hot water in Australia for supposedly misreporting the prospects for a new technique of genetic manipulation. The truth is more interesting and complicated than *Nature* (and many Australians) know.

Sydney

For the past month, *Nature* has been heartily reviled by many in Australia for the publication, on 12 October (**341,** 473; 1989), of a news report that an Australian patent application of which much was (and is still) expected had been anticipated by a patent application lodged earlier in the United States. The report has been especially hurtful to the Commonwealth Scientific and Industrial Research Organisation (CSIRO), which for the past decade, but especially in the past two years, has been under intense pressure from the federal government of Australia to win a greater commercial benefit from its substantial annual budget, but other officials of the federal government, ministers included, have also waxed indignant.

Further investigation, on a visit to Australia for other purposes, softens the assertion, in the report of 12 October, that an invention of which CSIRO, even Australia as a whole, is rightly proud has been fully anticipated by developments in the United States. To the extent that the report suggested that the Australian patent application is unambiguously pre-empted by the US patent claims, it is oversimple. But the relative status of the US and Australian claims is, to say the least, complicated and unclear. If the report had merely said (which it did) that the Australian claims will be fiercely disputed, it would have been correct.

Even so, *Nature* owes an explanation (unsolicited, as it happens) to those directly concerned — to CSIRO, to Dr Jim Peacock of the Plant Industry division of CSIRO, to Drs Wayne Gerlach and Jim Haseloff and to Mr Stephen Castle, a patent examiner at the Australian Patent Office (who was a casualty of the process of gathering the information on which the 12 October news report was based).

What follows is a circumstantial account of the origins of the disputed patents and of *Nature*'s two news reports on the subject (both that of 12 October and, earlier, on 3 August, **340,** 332; 1989). It is hoped that this account will be instructive (as well as interesting) for readers concerned about several features of the interaction between the scientific enterprise and the increasingly commercial environment in which it must make its way: the effects of commercialization on the open discussion of research; the extent to which publication policies are now affected by the consideration that patent rights should not be compromised; and the intricacies of patent law, with which researchers in many fields will have to become familiar.

The relationship between research and the news media is another of the issues that arises. In some ways, *Nature's* report lacked balance. But too little help was given by some of the respondents to its enquiries (although that is hotly denied).

Ribozymes

It is a shame that the research concerned, from which both the US and Australian patent applications stem, should be one of the neatest and potentially most important applications of genetic manipulation yet. Its origins are the recognition, within this decade, that intervening sequences ("introns") may in real life ("*in vivo*") be excised from freshly-transcribed RNA molecules to make functional molecules of messenger RNA (mRNA) without the intervention of extraneous enzymes — enzymes consisting of protein molecules, for example.

It was naturally a surprise, in the late 1970s, that it should have been discovered that genes of eukaryotic cells — stretches of double-stranded DNA helix — are not always connected pieces of helical DNA, and that functional pieces of DNA ("exons", where "ex-" stands for "expression") should be interrupted by apparently irrelevant stretches (called "intervening sequences", or IVSs).

Logic and cellular economy would argue that these irrelevancies should be irrelevant from the start, and thus never converted into RNA, but that is not what happens in real cells. Instead, the full length of a gene, introns as well as exons, is converted (transcribed) into a full-length molecule of RNA ("pre-mRNA") which is in turn converted into a molecule of mRNA somewhere in the nucleus. From the outset, this process (called splicing) has been a philosophical puzzle: for RNA transcripts to be accurately spliced, either the nucleus of a eukaryotic cell must contain a specification, distinct from the genetic DNA itself, of which stretches of RNA are to be excised, or the RNA transcripts themselves must contain a specification of how their intron sequences are to be removed.

In mammalian cells, many splicing processes turn on the recognition of splicing sites in RNA molecules by nuclear particles called small ribonuclear particles (RNPs). But it is now also plain that, for at least some introns, self-specified self-splicing is the rule. In other words, some introns embody both their own specification and the molecular mechanism by which they are excised. Dr Thomas Cech and his associates, of the University of Colorado at Boulder, have contributed substantially to this discovery in a series of articles in the international literature beginning in 1982. They have worked out the details of a mechanism by which introns are excised from RNA transcripts by means of intramolecular reactions between the nucleotides of the introns and those of the adjoining exons.

Among other things, Cech showed that some RNA molecules, entirely innocent of protein from which all previously known enzymes are made, can function as enzymes. He coined the term "ribozyme" to describe such enzymes consisting solely of RNA. The interest and importance of the discovery was recognized by the Nobel committees this year, which have awarded Cech the chemistry prize. The award is widely applauded. One member of the Canberra group whose work has prompted the Australian patent application says "we were astonished" by Cech's first evidence that introns could be excised from RNA molecules without the intervention of protein enzymes. Cech's Nobel prize, the

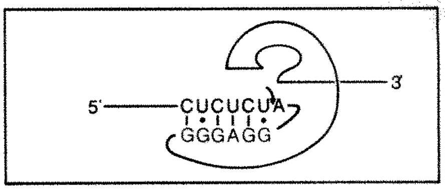

FIG. 1 Upstream splice recognition. The nucleotide sequence —CUCUCU is the end of the upstream exon, otherwise represented by the line labelled 5′. The nucleotide A is the first of the intron sequence, also represented as a line except for the six nucleotides —GGGAGG (reading from left to right), which are nucleotides 22 to 27 of the intron sequence in reverse order. Short vertical lines between pairs of nucleotides represent Watson–Crick (hydrogen) bonds between pairs of nucleotides. The directionality of polynucleotide molecules, whether RNA or DNA, is intrinsically determined by the way in which the linking phosphate groups can be attached either to the 5′ or to the 3′ position in the ribose (deoxyribose) ring of the sugar part of each nucleotide; because most, but not all, natural processes affecting polynucleotide molecules begin at the 5′ end, that is also called the upstream end or direction. From the Cech, Zaug and Been patent application.

patents examiners that their provisional judgements are mistaken.

The drift of the patent application rests squarely on the discovery, reported in January 1986, that the intron in the *Tetrahymena* pre-mRNA molecule can catalyse intermolecular reactions, and in particular carry out all the processes required for the removal of the intron, of which the most striking is the process by which an RNA molecule is cleaved at a predetermined sequence of nucleotides. The active ribozyme is the natural intron from whose 5′ end 19 nucleotides have been removed, and which is called L-19 IVS — L (for "linear"), "-19" (meaning "minus 19") and IVS (for "intervening sequence"). Figure 2 shows the functioning of this ribozyme in what may be its chief role as an RNA-restriction enzyme, or endonuclease.

The experimental conditions are important. Ions of Mg^{2+} are necessary, as is either guanosine (abbreviated G) or its phosphorylated derivative GTP. What then happens in self-excision is that, by a mechanism not understood, the RNA chain is broken after the recognition sequence of six nucleotides at the downstream end of the preceding exon.

Although the Cech, Zaug and Been patent application seeks protection for the enzyme functions of L-19 IVS other than its capacity to cleave RNA molecules — that of removing phosphate groups from the 3′ ends of RNA molecules, for example — they are less relevant to the Australian patent application and likely to be, in any case, less important. In the list of 73 claims in the Cech, Zaug and Been patent application, the second seeks protection for an "RNA enzyme" with "sequence-specific endonuclease" activity. Other more specific claims would protect the use of modified versions of the *Tetrahymena* ribozyme: the first two and the last of the 395 nucleotides may be eliminated from L-19 IVS without impairing its cleavage activity, while several examples are given of how the active (recognition) site of the enzyme can be modified so that it will cleave RNA molecules at places other than the join between a natural exon and an intron.

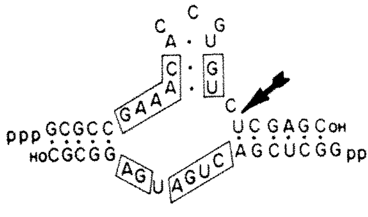

FIG. 4 Uhlenbeck's ribozyme. Two RNA strands synthesized by analogy with ASBV viroid sequences involved in a hammerhead self-cleaving region shown in the configuration in which they associate by means of Watson–Crick pairing. The lower strand is the 19-nucleotide ribozyme referred to in the text.

One question certain to arise in the months ahead is whether the claim for protection of endonuclease activity in the Cech, Zaug and Been application is so general that all further proposals for the use of ribozymes will be somehow subsidiary, at least in the United States (where Cech's prior publications are not, on the face if things, a bar to patent protection). The issue is not simply a matter of patent law and procedure; much will also depend on the commercial judgements of potential challengers of the relative costs of a challenge and a commercial compromise.

Further development

Apart from prompting a patent application, Cech's discoveries in the period 1982–86 seem powerfully to have stimulated other research groups already then concerned with the mechanism of self-splicing. One of these is the group of Robert H. Symons at the University of Adelaide, which has a long-standing interest in the mechanisms of splicing in RNA molecules, especially the RNA plant viruses and the RNA parasites of plants known as viroids and virusoids. Haseloff, one of the authors of the CSIRO patent application, was a member of this group as recently as 1982.

Symons and his associates appear to have succeeded in defining the necessary and sufficient structural requirements for self-cleavage in several of these plant RNA molecules. The results are neatly illustrated by the self-splicing of the virusoid of the lucerne transient streak virus (LTSV), which in its intact form consists of several virusoid particles, each a single-stranded RNA molecule 324 nucleotides long packaged, together with an RNA molecule ten times as long, into an icosahedral structure. The virusoids require the presence of the longer RNA molecule of the LTSV, and may themselves be formed as inactive rod-like particles of RNA in which the infective virusoid molecule is bound for most of its length to a Watson–Crick complementary "minus" strand. This rod like structure seems to be capable of splitting into two separate strands by self-cleavage (see Anthony C. Foster and R.H.Symons, *Cell* **49**, 211; 1987).

What Symons and his associates have shown is that both the plus and minus strands of the virusoid RNA (called vLTSV) are also capable of self-cleavage at specific places along their length, and that the sequence of nucleotides around that point will allow for a distinctive secondary structure of the RNA molecule, called graphically a "hammerhead" structure (see Fig. 3). Similar structures have been recognized in other plant virusoids, leading Symons and his colleagues to conclude that it is a structure prone to self-cleavage in which Mg^{2+} ions assist (but are not always necessary).

There is no suggestion that these hammerhead structures, recognized in RNA molecular parasites of plants, are involved in all RNA self-splicing; Cech's L-19 IVS is one exception; it is merely that the hammmerhead structure seems to be a sufficient condition for self-splicing in several natural RNA molecules. The work of Symons and his colleagues has been concerned exclusively with naturally occurring processes, and not with the design of molecules of RNA capable of acting independently as ribozymes in the strict sense.

Observations such as these show that a quite small stretch of RNA may be sufficient for self-cleavage, and it is natural that they should have helped to stimulate an attempt to define the minimum self-cleaving region. One important and relevant attempt in this direction is that of Ohlke C. Uhlenbeck from the University of Colorado at Boulder, a colleague of Cech's but not a member of his research group (see *Nature* **328**, 596; 13 August 1987).

Noting the demonstration by Symons and his colleagues of the effectiveness of hammerhead structures in the cleavage of plant parasite RNA, Uhlenbeck synthesized two RNA molecules with nucleotide sequences corresponding to those constituting the hammerhead structure in avocado sunblotch viroid (ASBV), showing that the shorter RNA molecule consisting of only 19 nucleotides would cleave its longer companion at the place predicted by what happens in ASBV itself (see Fig. 4).

That neat demonstration is likely to play an important part in the resolution of the status of the US and Australian patent applications, as the news report of the 12 October explained. Uhlenbeck's 19-nucleotide RNA is a true ribozyme, catalysing the cleavage of more than one molecule of its substrate. On the face of things, it appears also to be a particular example of the class of ribozymes at the core of the Australian patent application, which had not then been lodged. Although Uhlenbeck has not applied for a patent, and does not intend to do so (even under US law, he would be out of time), the publication of his paper may be held to invalidate later applications for patents covering ribozymes of similar design, or even to show that developments such as these were, by mid-1987, "obvious" in the sense in which patent law uses that term.

CSIRO's application

The Australian patent application, first lodged with the Australian Patent Office on 15 December 1987, is a model of clarity in such complicated circumstances. Observing that self-cleavage sites have been recognized in several plant viruses and related RNA, the document goes on to suggest a means by which efficient ribozymes may be constructed from synthetic RNA by an elaboration of the hammerhead principle.

Indeed, its authors generalize what is known of the common nucleotide structure of self-cleaving RNA molecules. Their claim rests on the assertion that ribozymes of any pre-determined specificity may be constructed by synthesizing RNA molecules in which the active cleavage element of the ribozyme is surrounded by short stretches of RNA capable of attaching, by Watson–Crick complementarity, to RNA sequences on either side of the desired cleavage site in the substrate RNA molecule (see Fig. 5). The essential elements of the patent application have been published (W. L. Gerlach and J. P. Haseloff, *Nature*).

Significantly, the Australian patent application (PCT/AU88/00478, published as WO89/05852) acknowledges Cech's earlier work and the existence of his patent application, but asserts that the two sets of ribozymes are distinct. First, the Australian application says, Cech's ribozyme occurs naturally in *Tetrahymena thermophila*. But the argument continues that Cech's ribozymes require the presence of guanine or one of its derivatives (usually GTP), add this nucleotide to the upstream end of the downstream cleaved fragment and holds that the Cech ribozyme is likely to be less specific than the Australian because fewer nucleotides can be involved in the hybridization of the ribozyme and its substrate molecule, especially because some of the crucial nucleotides cannot apparently be changed. The result, the Australian application says, is further to restrict the freedom to engineer specificity.

The Australian patent application is also more specific about the applications foreseen for artificial ribozymes than are Cech, Zaug and Been. In that document, the only use of ribozymes explicitly described is as laboratory reagents. But the Australian patent application says that ribozymes can be engineered to cleave any RNA molecule at a pre-determined sequence of ribonucleotides, meaning that target RNA sequences can be made inactive. Alternatively, as the case is put, ribozymes might be produced endogenously in cells by the transfer of self-replicating plasmids or by microinjection.

The application says explicitly that infectious mammalian viruses might be inactivated by the direct administration of appropriate ribozymes or by incorporating into vectors such as vaccinia virus carrying sequences of DNA, that would allow the production of the ribozymes endogenously. The same (the application says) could be done to neutralize the effects of unwanted gene activity in plants, even to the extent of engineering plants that produce fruit without stones, as well as to treat genetic disease in people where the underlying defect is the overproduction of a protein produced at the prompting of some mRNA.

Nobody can cavil at the importance of these claims. If CSIRO and its commercial partners — CSIRO hopes there will be an Australian partner as well as the French company Groupe Limagrain — can find a way of delivering specific ribozymes into misfunctioning cells, they will have found a way of curing many of the world's ills. There may be practical difficulties to be overcome before engineered ribozymes can be used as medicines, and regulatory hurdles to be surmounted before vaccinia virus and other genetic vectors can be used in the prophylaxis of human disease, but CSIRO's enthusiasm for its "gene shears" process should not be crabbed.

Disputations

Nobody, unfortunately, can tell in advance how patent lawyers and (if it comes to that) the courts will determine the relative merits of these rival, but not necessarily conflicting, claims. Cech, Zaug and Been were first, by more than two years, but Gerlach, Haseloff and Jennings describe what appears to be a more efficient ribozyme, and one whose specificity may be pre-determined by a knowledge of the nucleotide sequence of the RNA it is meant to cleave, irrespective of its source.

Variations of patent law from one country to another will complicate the resolution of the issue; for example, patents might be awarded in the United States to both applicants, but to one or other elsewhere. On the face of things, the existence of independent prior publications, such as those of the Symons group and particularly of Uhlenbeck, would seem to be an embarrassment for CSIRO's case. On one view, the commercial value of CSIRO's gene shears technique may hang on the speed with which the organization is able to work towards its practical exploitation.

How did *Nature* come to be calling the odds on such a complicated issue in its news report of 12 October? It made mistakes, listed in what follows, in incompletely extenuating circumstances.

One error was that of CSIRO, which first annouced at the end of July this year that it had signed an agreement with Groupe Limagrain for the exploitation of its "patent" on gene shears. If that claim had been less definite (only an application for a patent had then been lodged), *Nature*'s informants in the United States might have been less eager to say that something must be wrong. The outcome was a conversation with the Boston-based patent attorneys acting for US Biochemicals Corporation, which has acquired rights to exploit the Cech, Zaug and Been patent.

Six weeks went by before *Nature*'s Australia correspondent, Tania Ewing, who had by then spoken to the US patent attorneys, approached Dr Jim Peacock, at the Plant Industries Division of CSIRO, telling him at the outset only that doubts had been cast on CSIRO's announcement in July, but in later conversations asking for his reaction to the US claims that the Cech patent application had precedence over whatever might be claimed in Australia. The response appears to have been unspecific; detailed discussion was declined, but Dr Peacock and other members of the gene shears team insisted that they were "confident" that their patent application would succeed, pleading CSIRO's patent lawyers in their support. But access to the lawyers was denied, on the grounds that "that is not CSIRO policy".

The contemporary sociology of Australia is relevant. The country is lavishly over-governed, but the press is constitutionally powerful; public officials who refuse to answer questions on the telephone may be lampooned. Meanwhile, CSIRO has made a painful transition from semi-autonomous research organization to government agency of a kind; the pressure to succeed commercially is intense and insistent, as is the fear of failing to succeed. So researchers dutifully answer the telephone, even returning calls from journalists, but deal cautiously with questions of a kind that politicians might understand.

If Tania Ewing had been offered a sight of the two disputed patent applications, she would not have concluded that her US patent attorney informants were correct in their assertion that the Cech patent would supervene and that CSIRO's confidence was misplaced; instead, she would probably have decided that there is a tangled fight ahead. Yet even two weeks ago, CSIRO was still saying that it would be possible to secure a copy of the Cech patent application only from the International Patent Office at Geneva, its own complement of photocopying machines notwithstanding.

Those, so far as *Nature* is concerned, are most of the extenuating circumstances. Three errors in the 12 October account stand out, as follows:

- Dr Jim Peacock was at one stage asked whether he "remembered a letter from a man called Jim Chen", allegedly a discouraging report on the Australian patent application from the Australian Patent Office. But in reality, the document concerned was not a letter but a standard form report (citing the Uhlenbeck research as potentially prior art), with Jim Chen's signature in the bottom right-hand corner, Peacock had indeed forgotten (if he had ever remembered) that the form had been signed by Chen. When reported to have "forgotten," the results of this international patent search, he was made to look a fool. He was, of course, fully aware that he had received the research report.

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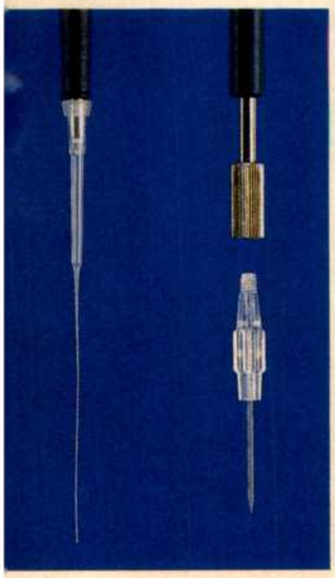

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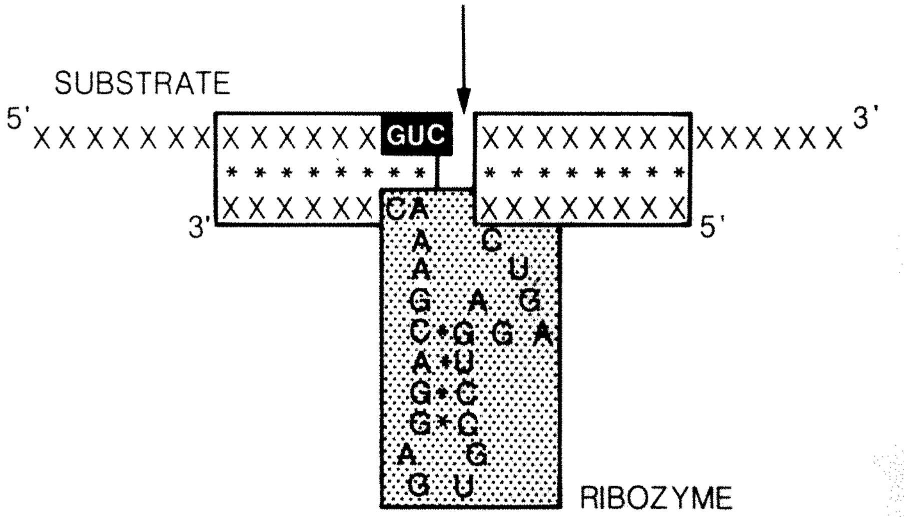

FIG. 5 The CSIRO ribozyme. Schematic diagram (from the Australian patent application) in which X stands for any nucleotide and X.X for any (or either) pair of complementary nucleotides. The shaded block includes the shortest RNA sequence found to be effective in cleaving RNA parasitic on plant cells. As shown, the ribozyme would cut the upper substrate RNA strand after the nucleotide triplet GUC, but the patent application argues that this restriction may be relaxed so long as the central nucleotide in the triplet remains as uracil and so long as the third is not guanine. The RNA sequences attached to the common ribozymal unit would be synthesized so as to be complementary in sequence to the naturally occurring substrate and thereby enhance the specificity of the ribozyme.

■ In the same vein, Mr Stephen Castle at the Australian Patent Office was quoted directly as saying "We don't think they [CSIRO] will get their patent". There is a direct conflict of evidence on this point between what Mr Castle says occurred and Tania Ewing's notebook. Mr Castle says that on the day Ms Ewing reached him by telephone, he was standing in for his immediate supervisor, Jim Chen. The question was whether the "X" rating in respect of Uhlenbeck's paper would be fatal for CSIRO's application. Mr Chen says that he explained he had no direct knowledge of the application, that he explained that he could not discuss particular applications, but recalls saying that such an annotation "means that a patent will probably not be granted". At an interview conducted in the presence of Mr Murray Heddrich, Deputy Commissioner of the Patents Office, Mr Castle also says that he explained that an "X" document would invalidate only the particular claims to which the annotation referred (a third of the total in the case of the Australian patent application) and that applicants might in any case successfully challenge the examiner's judgement.

There is no simple way in which such a conflict between written and remembered evidence can be reconciled. But it can be understood. In Ms. Ewing's notebook, the quotation "We don't think they'll get their patent" follows directly after a notation of a question about Uhlenbeck's article, implying that the two questions were closely linked. Mr Castle's recollection is that they were widely separated. Whatever the intervening material, it goes without saying that anybody hearing the phrase "We don't think they'll get their patent", even if in response to a question about the general significance of "X" ratings, would have concluded that what applies to the general also applies to the particular, only recently discussed. But Mr Castle's reservations, however emphasised during that conversation still apply: prior publications threaten only the claims to which they are judged relevant, and that judgement may always be challenged. The moral seems to be that, when writing about patent applications, it is important to read them (if you can get hold of them).

■ In the United States and Western Europe, *Nature* distributes each week to interested journalists a summary of each week's scientific content, regretting that the timing of this operation is such that important items of news cannot be thus advertised. On this occasion, it was agreed between Sydney and Washington that advance notice of the news report of the gene shears patent should be distributed to a few Australian journalists, which had the unfortunate effect of amplifying the misleading aspects of the news report.

The first of these issues is a misunderstanding, the second a potential misunderstanding, the third is an error of judgement. *Nature* is sorry for the hurt they may have caused, however trying the circumstances. But it is hard to fathom the ferocity of the Australian reaction. Mr Barry Jones, the federal Minister of Science, telephoned Ms Ewing on the afternoon of 19 October to say that the Australian government would sue her and *Nature* for what she had written, which information was the basis for at least two daily newspaper reports the following day. Two weeks ago, Mr Jones rejected the charge that he was seeking to intimidate journalists, but could not recall the circumstances in which he had made the call.

Nature has the fullest confidence in its Australia correspondent and hopes to be able to do so for many years. There are nevertheless several lessons to be learned from this saga, as much by people in offices in Washington and London as by reporters in the field. One is the old journalistic saw that one cannot be too careful — of volunteered information (although, without it, journalism would grind to a halt), of the contents of documents which have not been read, but only reported by potentially interested parties, and of the difficulties of pursuing information on two continents which are separated by 16 hours on the clock. On this occasion, proper scepticism of Australian claims seems to have been unfairly magnified.

The plain truth is that the Gerlach and Haseloff design for ribozymes is certain to be widely applied in the laboratory processing of RNA, while the potential of the applications in medicine and agriculture is potentially very great. Before these prizes can be won, of course, several less interesting practical problems will have to be solved. Using ribozymes as medicines for the treatment of AIDS, for example, will require that means of delivering effective doses of the material to infected lymphocytes will have to be found. By all accounts, the pharmaceutical companies are not amused.

Yet Peacock says that the collaboration with Groupe Limagrain has already embarked on two development projects with promise. It would be good to think that Australia will yet win a good share of the benefits seemingly in sight. The pressure exerted by the federal government in such directions may often be over-onerous or even short-sighted, but it has been Australia's dream for several decades to supplement its staple exports of primary products from mines and farms with exports whose added value is greater. That, of course, is why neat gene shears were so generally acclaimed in July this year.

The question whether interesting science is now too much confused with the patents business is a perennial question but also often an academic one. For many people there is often no choice. Protecting intellectual property has become part of research, even survival in research. Yet the task of briefing patent attorneys is an irksome and time-consuming obligation from which some people still shrink. A predictable but unexpected consequence is the effect of the patents business on people's demeanour. Much of the reticence encountered in describing CSIRO's gene shears seems to have arisen both from unfamiliarity with arcane patents practices and from the way in which attorneys frequently swear their clients to silence. Too much of that could bring great trouble. □

VOLCANOES

Magma transport through dykes

Greg A. Valentine

THE transport of magma through the Earth's crust, whether from relatively shallow magma reservoirs (depths of a few kilometres) or the upper mantle (depths approaching 100 km) has always presented geologists with a challenging problem. Much of the challenge arises from the complex behaviour of magmas under conditions of changing temperature, pressure and composition. An important aspect of magma flow through fissures is the exchange of heat with the surrounding rocks. For flow to be sustained, the heat advected from the magma reservoir must successfully compete with the loss of heat to the cooler country rocks. This competition leads to a physical system in which hydrodynamics (the advection of heat) and thermodynamics (latent heat from solidification and diffusion of heat into surrounding rocks) are strongly coupled and cannot be treated as independent processes. In an important new analysis, Bruce and Huppert show on page 665 of this issue[1] how the coupling can lead to complex processes of solidification followed by re-melting. They derived a criterion that separates dyke blockage by solidification from sustained flow.

A typical basaltic fissure eruption, on Mauna Ulu, Hawaii, 1969, showing fire-fountaining due to eruption of gas-rich magma. These eruptions commonly occur along an entire fissure during initial phases, but eventually become localized to a few discrete vents along the fissure. According to Bruce and Huppert[1] this trend may be the result of extreme sensitivity of magma flow in fissures to the initial width of the fissure. Small irregularities in the initial width may become pronounced as an eruption progresses, leading to concentration of magma discharge in slightly wider parts of the fissure. (Photograph by W.A. Duffield, US Geological Survey.)

Field evidence shows that magma is transported through the crust mainly by the flow of magma through cracks, or dykes. Hence, dykes are a significant feature in nearly all magmatic provinces of all ages, especially where mafic or ultramafic (iron- and magnesium-rich) magmas are present. In areas undergoing tectonic extension, such as mid-ocean ridges and continental rifts, the dominant means of magma transport to the surface is within dykes. Oceanic islands within tectonic plates, such as Hawaii, are characterized by large, low-relief shield volcanoes that erupt magmas from linear fissures on their summits and flanks (see figure); these fissures are the surface expression of dykes. Even silicic magmas are commonly influenced by flow in dykes at some level in the crust. Examples of this include dykes that radiate out from central conduits of stratovolcanoes, linear sytems of silicic domes and ring dykes associated with large intrusions.

Magma transport via dykes presents two problems which can, to some extent, be treated separately. The first is formation of the crack. In many cases the initial crack may be a pre-existing fracture in the host rock, but in others the fracture is probably propagated by the magma itself in a manner similar to hydrofracture processes. This magma-fracture process has been studied by Spence and Turcotte[2] for highly simplified cases that can be mathematically analysed by a similarity solution. They find that magma can propagate fractures in a vertical direction at speeds of the order of $0.01-100\ \mathrm{m\ s^{-1}}$, the buoyancy of the magma driving them upward. Their conclusion is that most of the transport problem resides in the flow of magma once the fracture has opened; the initial fracturing itself is not, in general, a limiting process.

The fluid-flow problem is more daunting owing to the nature of magmas. The rheology of magma is strongly dependent upon its composition. Viscosity, for example, can vary over several orders of magnitude for a change in SiO_2 content of just 20 per cent. Furthermore, at any given composition, the viscosity depends on the temperature and strain rate. As a magma cools or heats, it can undergo drastic phase changes that modify the behaviour of the fluid and that involve significant exchanges of latent heat. Previous treatments of magma cooling due to heat loss to the dyke walls have consisted of simple static conduction calculations[3], empirical calculations of heat exchange between flowing fluid and a wall for one-dimensional flow[4], and two-dimensional calculations allowing for some advective effects[5]. None of these formally allowed for the effects of latent heat released or absorbed by material as it solidifies or melts, respectively.

Bruce and Huppert[1] make an important step in the theoretical treatment of magma flow in dykes by allowing the advection of heat by flowing magma to affect the temperature distribution in the wall rock. They include melting, solidification and related latent-heat effects in their analysis. Two important regimes are found: one in which heat loss to the walls exceeds heat advection and the dyke freezes shut; and a second in which initial heat loss may exceed advection, but in which advection and latent heat effects eventually win and the dyke remains open and may even grow wider. This second regime involves initial solidification of magma along the walls followed by re-melting (meltback).

Bruce and Huppert's calculations have a wealth of implications for eruption dynamics and petrological variations of eruptions from fissures. For example, the sequence of initial solidification followed by meltback could strongly influence eruption dynamics. The solidification would concentrate volatiles into the remaining melt in a dyke, which upon eruption may appear to be 'artificially' volatile rich. In a basaltic fire-fountain eruption, this partitioning of volatiles may lead to a higher fountain, which would in turn lead to overestimates of the volatile content in the magma chamber. Later, as meltback occurs, volatile-depleted material (the old chilled margin) would be mixed up with the flowing magma to deplete the eruptive mixture 'artificially'. Such events could produce apparent variations in original magma composition, even if the original composition was unzoned. Is it possible that this process is responsible for fluctuations in fountain heights during eruptions such as observed by Head and Wilson[6]?

These processes could also influence the major- and trace-element composition of erupting magmas, which should be altered to some degree by the solidification–meltback process, especially if meltback reaches past the original dyke walls and involves original country rock. Also, they are likely to produce nonequilibrium textures in phenocrysts.

It seems essential to collect samples of basalts as they erupt from a given vent, in order to observe some of the processes that Bruce and Huppert predict, as cooled dykes are highly unlikely to preserve these processes. A difficulty arises because the original compositional zonation in the magma reservoir and the effects of draw-up into the dyke would have to be established independently of the basalt sampling — a challenging prospect.

Finally, it is worth noting that even though Bruce and Huppert's analysis is an important step in elucidating magma transport in dykes, we are still a long way from a physically complete understanding of the process. For example, none of the analyses mentioned above, including Bruce and Huppert's, takes into account the real rheology of magmas. In addition, multiphase flows[7] that result from the release of dissolved gas from the magma need to be incorporated into the heat-transfer problem. Neglect of these processes is attributable partly to poor data on magma properties, but also to limitations in mathematical approaches that rely mainly on analytical techniques. Numerical solutions of the full set of governing equations with minimal simplifications would constitute a significant advance. ☐

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1. Bruce, P.M. & Huppert, H.E. *Nature* **342**, 665–667 (1989).
2. Spence, D.A. & Turcotte, D.L. *J. geophys. Res.* **90**, 575–580 (1985).
3. Wilson, L. & Head, J.W. III *J. geophys. Res.* **86**, 2971–3001 (1981).
4. Spera, F.J. *Contrib. Mineral. Petrol.* **88**, 217–232 (1984).
5. Delaney, P.T. & Pollard, D.D. *Am. J. Sci.* **282**, 856–885 (1982).
6. Head, J.W. III & Wilson, L. *J. geophys. Res.* **92**, 13715–13719 (1987).
7. Vergiolle, S. & Jaupart, C. *J. geophys. Res.* **91**, 12842–12860 (1986).

CELL BIOLOGY

The pushy ways of a parasite

John E. Donelson and Alice B. Fulton

A SERIES of electron micrographs in the *Journal of Cell Biology*[1] provides new insight into how an intracellular bacterial parasite, *Listeria monocytogenes*, behaves inside cultured macrophages and how it moves from one macrophage to another without ever leaving the cytoplasm. The authors of the paper, Tilney and Portnoy, not only demonstrate the cunning way in which the parasite traverses the perilous divide between host cells, but provide pointers to fresh approaches for studying the cytoskeleton.

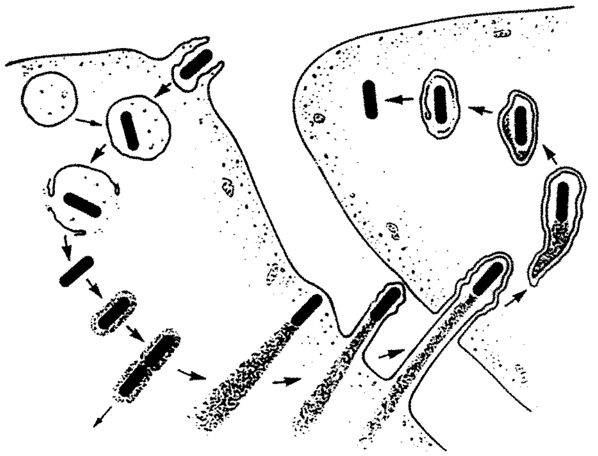
All parasites of mammals must evade the immune systems of their hosts. Some do it by hiding within host cells, but this strategy has its own complications as the insides of host cells can be distinctly hostile[2]. Parasites that survive within host cells such as macrophages have therefore evolved a variety of mechanisms to prevent themselves from being destroyed by cellular defences such as lysosomal acid hydrolases and the oxidative response. For example, some intracellular parasites inhibit fusion between the parasite-containing phagosome and lysosomes *(Toxoplasma, Chlamydia);* others are resistant to lysis by lysosomal enzymes (*Leishmania*, mycobacteria); and still others escape from the acidic 'phagolysosome' into the host cell's cytoplasm *(Listeria, Trypanosoma cruzi)*[3]. But even after coping with the host cell's defences, the parasite must still avoid destruction by the humoral immune system during transfer from one cell to another.

Listeria monocytogenes is a gram-positive, non-spore-forming pathogen of humans, sometimes found in unpasteurized milk, that penetrates macrophages, fibroblasts and enterocytes. Following phagocytosis, the parasite-containing endosome fuses with one or more lysosomes (see figure). Tilney and Portnoy's electron micrographs show that the phagolysosome membrane dissolves before the bacterium is damaged; the membrane is probably degraded by a bacteria-encoded haemolysin, because *Listeria* mutants lacking haemolysin fail to escape from the phagolysosome[4].

After phagolysosome breakdown, the *Listeria* subverts the macrophage's cytoskeleton for its own purposes in an unexpected way. First, a halo of actin filaments (which are decorated by myosin subfragment-1) surrounds the bacterium. After the *Listeria* divides, this halo becomes asymmetric, forming a tail at one end of the bacterium and taking on the appearance of a short comet. The halo and comet tail are densely packed with short randomly ordered filaments. As the tail elongates, the bacterium is apparently 'pushed' by the comet tail towards the macrophage surface. Once at the surface, the *Listeria* distends the host membrane to form a pseudopod-like extension that touches another macrophage. The *Listeria* and some of the actin tail are engulfed by the new host cell and another round of infection begins. Thus, once safely inside the first macrophage, the *Listeria* need not confront the perils of the humoral immune system again.

This exploitation of the host's cytoskeleton raises several questions. First, how does the *Listeria* induce the macrophage's actin to polymerize? The halo and comet tail are not simply pre-existing filaments recruited into these structures, because cytochalasin D prevents their formation and stops the bacterium from moving towards the surface. The filaments appear to grow from some component of extracellular material around the *Listeria* as all the filaments begin near the bacterial surface. *Listeria* has recently been found to export a basic (isoelectric point 9.3) protein of relative molecular mass 60,000 involved in bacterial attachment to the host cell[5]; perhaps this positively charged protein also nucleates actin polymerization in the host cytoplasm, as does polylysine *in vitro*[6].

Second, why are these particular filaments so short, skewed and stable? Their average length appears to be only a few tenths of a micrometre, yet the comet tail of several micrometres is stable for up to an hour. Does *Listeria* secrete a toxin, such as



Steps in the entry, multiplication, movement and transfer of *Listeria* from one macrophage to another. Once inside a macrophage the bacterium escapes into the host's cytoplasm and is surrounded by a halo of the host's actin. After division, the actin around the daughter bacterium becomes asymmetric, taking on the appearance of a comet. As the tail elongates, the bacterium is apparently propelled towards the macrophage membrane, where a pseudopod-like extension of the cell membrane is formed. When the pseudopod is engulfed by another macrophage, the bacterium is released into its new host cell.

phalloidin, that stabilizes filaments? If so, how does the rest of the actin in the cell escape its effects? Why is the long comet tail composed of these short, skewed filaments when normal elongated actin structures generally have longitudinal order, sometimes approaching a paracrystalline state? Does *Listeria* secrete a bundling protein to hold together the short, skewed filaments, or does it recruit one from the host?

Perhaps the simplest explanation is that *Listeria* has many high-affinity nucleation sites for actin polymerization on its surface. The progeny bacterium appears to have fewer sites at one end so that actin polymerization imparts motion to the bacterium by mechanically pushing it. Actin polymerization is known to be involved in pushing out the membrane of the acrosomal process of *Thyone briareus* sperm[7].

Regardless of the mechanism underlying the bacterium's movement, how it becomes directed towards the macrophage surface remains unexplained. However, if the nucleating component is shed by the bacterium as it moves, the filaments would be left behind in the tail. The apparent stability of the filaments may be a consequence of their exceedingly high density and the presence of the nucleating component. Their disarray may reflect the absence of crosslinking proteins.

At least some of these questions can be addressed at the genetic level because transposon mutagenesis and plasmid DNA transformation can be carried out with *Listeria*[8]. Furthermore, cytoskeletal subversion may be a common tactic used by other intracellular parasites. Intracellular infections by bacterial and protozoan parasites have long been used as models for studying cell-mediated immunity — Tilney and Portnoy's findings indicate that they are also good systems for examining cytoskeletal formation, organization and function. ☐

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1. Tilney, L.G. & Portnoy, D.A. *J. Cell Biol.* **109**, 1597–1608 (1989).
2. Moulder, J.W. *Microbiol. Rev.* **49**, 298–337 (1985).
3. Sher, A. & Colley, D.G. in *Fundamental Immunology*, 2nd edn (ed. Paul, W.E.) 957–983 (Raven, New York, 1989).
4. Portnoy, D.A., Jacks, P.S. & Hinrichs, D.J. *J. exp. Med.* **167**, 1459–1471 (1988).
5. Kuhn, M. & Goebel, W. *Infect. Immun.* **57**, 55–61 (1989).
6. Lin, D.C., Tobin, K.D., Grumet, M. & Lin, S. *J. Cell Biol.* **84**, 455–460 (1980).
7. Tilney, L.G., Bonder, E.M., Coluccio, L.M. & Mooseker, M.S. *J. Cell Biol.* **97**, 112–124 (1983).
8. Gaillard, J.L., Berche, P. & Sansonetti, P. *Infect. Immun.* **52**, 50–55 (1986).

PALAEOCLIMATE

Deep trouble for climate change

N.J. Shackleton

THE search for geological evidence of past climate changes has yielded much material for modellers trying to understand the principal factors controlling these changes, be they variations in the ocean–atmosphere system or slow changes in the Earth's orbit as suggested by Milankovitch. New data derived from the ages of fossil corals, presented by Fairbanks on page 637 of this issue[1], show that we still do not understand the very rapid change in climate during the period known as the Younger Dryas at the end of the last glaciation.

The fossil coral terraces of tropical islands provided the first convincing geochronological evidence that the Milankovitch hypothesis of long-period climate forcing might be correct[2]. The uplifted coral tracts have yielded extremely valuable data on the history of climate-induced changes in sea level[3,4]. Coral deposited over the past few thousand years has also been dated for reconstructions of the approach of sea level to its present position as the ice sheets of the last glacial maximum finally faded away[5]. By drilling into the uplifted interglacial terraces in Barbados, Fairbanks and Matthews[6] were able to document on the ground the relationship between the changing isotope composition of the oceans as ice sheets melted and grew, and the height of the sea relative to Barbados.

Despite these contributions, there has been a fundamental difficulty in interpreting the ocean oxygen-isotope record — a key to so many aspects of palaeoclimatology — because we have had no means of accurately comparing ^{18}O and ice volume, or sea level, over the whole glacial–interglacial range[7]. Armed with his experience of the uplifted coral terraces, Fairbanks has now filled in much of this gap in our understanding by taking a look at the submerged coral, which one had always suspected must be there awaiting attention. In this issue[1] he shows the first, exciting results of that enterprise.

What Fairbanks has done is to drill and core submerged coral at depths below sea level ranging from 10 m to about 130 m. Concentrating on *Acropora palmata*, which grows only within about 5 m of the sea surface, he has obtained the ^{14}C age and present depth of each piece. Correcting this depth for the slight uplift of the island over the past 15,000 years he obtains the first almost continuous record of sea-level rise during deglaciation (even a single well-dated estimate of the glacial low-stand would have justified publication).

Still more exciting is the fact tha Fairbanks's temporal cover is good enoug for him to differentiate the sea-level curv to present a direct measure of the rate o rise of sea level, which is of course equivalent to a measure of the meltwater flu into the oceans. As Fairbanks points out this will be subject to some subtle modification when we have a more accurat knowledge of the relation between so-called 'radiocarbon years' and true years However, his conclusions, even as the stand, call into question a recent interpretation of the Younger Dryas event.

The Younger Dryas event was an interva lasting a few centuries between 11,000 an 10,000 years before present (BP) durin which the climate of northwest Europe which had been quickly improving afte the glacial maximum, was suddenly throw back to near-glacial levels. Glacier advanced again, an event marked as the Loch Lomond Stadial in Britain. The reason for this event has intrigued people for many years; one reason for devoting special attention to it is that whereas the growth of the great ice sheets took place over thousands of years, this event appears to testify to the ability of the global climate system to shift from an interglacial to a glacial state in a matter of decades. Is global climate balanced so finely that it could flip easily into a glacial mode?

In a series of papers[8,9], Broecker has argued that the Younger Dryas may have been a feature peculiar to the deglaciation period rather than something that could happen at any time. He recently demonstrated[8] that the Younger Dryas occurred between two periods during which glacial meltwater flooded down the Mississippi river to the Gulf of Mexico, as indicated in cores recovered from the Orca Basin. Broecker's argument is that, between these two events, meltwater was diverted and instead entered the ocean so far north that it upset the density stratification of the North Atlantic.

His reasoning is that such a huge meltwater influx down the Saint Lawrence Seaway would significantly lower the salinity of the surface of the whole northern part of the North Atlantic. This would prevent the winter cooling of the sea surface in the Norwegian and Labrador Seas from raising the density of the water enough that it could sink to form the North Atlantic Deep Water. Broecker's underlying hypothesis[9] is that the global climate system has two stable states, a glacial state and an interglacial state, and that upsetting deep-water formation in the North Atlantic is a mechanism for flipping the system into the glacial state. In this picture, the Younger Dryas would be such a flip into the glacial state that arose as a chance by-product of the geographically complex melting history of the North American ice sheet.

Fairbanks's work shows that, in fact, melting almost stopped during the Younger Dryas, the meltwater flux fell to a fifth of the maximum value it had had a millennium previously The peak flux was at 12,000 years BP, identical in age with the greatest Mississippi flow[8] By 11,000 years BP, the rate of sea-level rise was at a minimum, the same date as the cessation of enhanced Mississippi flow Fairbanks's data show that the pulse of very rapid melting came to an end shortly after 12,000 BP, it would be very difficult now to argue that the diversion of the Mississippi flow could have been the main trigger for this important climatic episode, and it seems likely that the whole edifice that Broecker elegantly erected to explain it has collapsed like a house of cards At the same time the climatic importance of the event seems to be reinforced, as it did, indeed, almost totally halt the dramatic retreat of the great ice sheets Doubtless Broecker will reconstruct his hypothesis even more elegantly in his next contribution □

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1 Fairbanks, R G *Nature* **342**, 637–642 (1989)
2 Mesolella K J Matthews R K Broecker W S & Thurber, D L *J Geol* **77**, 250–274 (1968)
3 Matthews, R K *Quat Res* **3**, 147–153 (1973)
4 Bloom A L Broecker W S Chappell, J M A , Matthews, R K & Mesolella, K J *Quat Res* **4**, 185–205 (1974)
5 Lighty R G McIntyre I G & Stuckenrath, R *Coral Reefs* **1**, 125–130 (1989)
6 Fairbanks R G & Matthews R K *Quat Res* **16**, 181–196 (1978)
7 Shackleton N J *Quat Sci Rev* **6** 183–190 (1987)
8 Broecker, W S *et al Nature* **341**, 318–320 (1987)
9 Broecker W S Peteet D M & Rind D *Nature* **315** 21–26 (1985)

MHC PROTEIN STRUCTURE

Getting into the groove

Peter Parham

In October 1968, purified papain-solubilized HLA-A2 made its debut in the pages of *Nature*[1], nineteen years later the crystallographic structure of this human class I major histocompatibility complex (MHC) molecule provided dramatic insights into T-cell biology and laid the ground rules for future experiments and speculation[2,3] Perhaps of greatest impact was the 'extra electron density' on display — the hazy, ill-defined image of something filling the binding site, with far-reaching implications for antigen presentation, MHC restriction and T-cell tolerance From the genetic perspective it was their diversity and polymorphism that drew attention to MHC proteins, and although modelling of many MHC sequences into the A2 structure indicated that residues lining the sides and floor of the peptide-binding groove were the foci for advantageous amino-acid substitution[2–4], little was revealed of the architectural details resulting from this diversification of the antigen recognition site With refinement of HLA-A2 and solution of the structure of HLA-Aw68, Garrett *et al* [5] now begin to provide such insight on page 692 of this issue they guide us through the interstices of the celebrated 'groove', and in so doing complete what is arguably the first crystallographic experiment in MHC genetics

Polymorphic pockets for peptides. It is the extracellular region of class I molecules that binds peptides, T-cell receptors and either the CD4 or CD8 coreceptor[6] These molecules consist of two immunoglobulin domains and two 'peptide-binding' domains For HLA-A2, HLA-Aw68 and other class I MHC molecules, the polymorphic and membrane-anchoring heavy chain contains both peptide-binding domains (α_1 and α_2) and one immunoglobulin domain (α_3), while a second, and in humans invariant, polypeptide (β_2-microglobulin) provides the second immunoglobulin domain[2] In contrast, class II molecules have a symmetrical, and probably more ancient, arrangement in which two membrane-spanning polypeptides each contributes one peptide-binding and one immunoglobulin domain

With minor perturbations, the polypeptide backbones of HLA-A2 and HLA-Aw68 are extremely similar[5] This greatly simplifies comparison of the two structures for the differences between them are primarily due to the nature of amino-acid side chains at 13 positions (six in α_1, six in α_2 and one in α_3) of substitution in the heavy-chain sequences (Fig 1*a*) Ten of these residues are at positions lining the floor and sides of Garrett's groove and the differences in their chemistry, size and polarity have profound effects on its contours and on the peptides that bind, as is vividly shown by differences in the extra electron density of the two crystals

Although the antigen-recognition site (α_1 plus α_2) is symmetrical, the disposition of substitutions between A2 and Aw68 is not A 'boomerang' pattern of variation, extending along the α_1 helix and then traversing the β structure of α_2 to the centre of the α_2 helix (Fig 1*b*), is in fact similar to the distribution of high-variability residues obtained from accumulated HLA-A,B,C sequences[7] The reason for such segregation of variation and conservation within the binding groove is not discerned by Garrett *et al* [5]

a

		▼		▲	▼	▼	▼	▼	▼	▼			▼	▼	▼	
		9	12	62	63	66	70	74	95	97	105	107	114	116	156	245
				+									+			
Aw68	(Aw68 1)	Tyr	Val	Arg	Asn	Asn	Gln	Asp	Ile	Met	Ser	Gly	Arg	Asp	Trp	Val
	Aw68 2	—	Met	—	—	—	—	—	—	Arg	Pro	—	His	Tyr	—	—
	Aw69	—	—	—	—	—	—	—	Val	Arg	—	Trp	His	Tyr	Leu	Ala
A2	(A2 1)	Phe	—	Gly	Glu	Lys	His	His	Val	Arg	—	Trp	His	Tyr	Leu	Ala
					−	+	(+)	(+)		+			(+)			
								Δ		Δ				Δ		
		← α_1 →							← α_2 →							← α_3 →

b

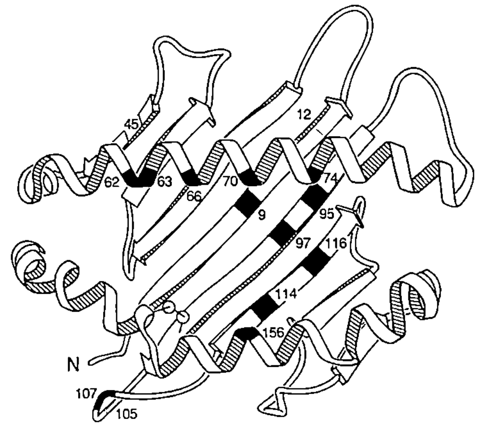

FIG 1 *a*, Amino-acid substitutions between A2, Aw68 and related molecules Sequences are compared to Aw68 1 and only positions of difference are shown identities with Aw68 1 are given by lines The ten substitutions between A2 1 and Aw68 1 that line the peptide-binding groove are indicated by (▼) and the substitution at 62 that is hypothesized to affect T-cell receptor contacts is given by (▲) Residues affecting the 74 pocket are indicated by (Δ) Charged residues are noted by plus or minus Histidines, a characteristic of A2, are indicated with (+) as their protonation under physiological conditions is uncertain

The nomenclature for human class I MHC molecules is confusing because names have traditionally been given on the basis of serological reactivities which often encompass the products of more than one allele Thus serologically defined HLA-A2 is known to include at least nine distinct proteins of which the 'A2' structure[2,3] derives from HLA-A2 1 Serologically defined HLA-A28 was initially divided into HLA-Aw68 and HLA-Aw69, and subsequently HLA-Aw68 into HLA-Aw68 1 and Aw68 2 (Garrett *et al* 's structure is of Aw68 1) Revision of nomenclature in terms of sequence is fortunately in progress

b, Schematic representation of the α_1 and α_2 domains of the HLA-A structure showing the 'boomerang' pattern of substitutions Differences between A2 1 and Aw68 1 are shaded solid, substitutions specific to Aw68 2 are shaded with stipple Position 45, which is invariant in HLA-A sequences and contributes to the conserved 45 pocket, is also shown (Adapted from Fig 2 of Bjorkman *et al* [2])

HIGH-ENERGY PHYSICS

The Big Bang's magic number

Frank Close

INITIAL data from the LEP electron–positron collider at CERN, Geneva, now formally published in *Physics Letters*[1-4] confirm one of the cornerstones of modern physics, namely the Big Bang model for creation of matter

The Big Bang model rests, in large part, on the ability of theory to explain the relative abundances of the light elements, in particular the primaeval ratio of hydrogen to helium The fusing of these light elements took place during a brief interval in the early Universe Before this time the heat was too great for the nuclei to stay bound, beyond this time free neutrons had decayed and were no longer available for the alchemy The elemental abundances depend, *inter alia,* on the amount of time the Universe spent in these judicious conditions, which depends on its rate of expansion and this, in turn, depends on the number of light particle varieties around — hence on the number of light neutrinos Cosmologists had thereby determined that they could explain the elemental abundances if there were at most four varieties, or 'flavours', of light neutrino, where light means less than about 1 MeV (that is less than about twice the mass of the electron)

The LEP results reveal the number of light neutrinos directly, without any assumptions about elemental abundances or cosmology The essential key is that counter-rotating beams of electrons (e^-) and positrons (e^+) annihilate at an energy tuned such that they produce the massive Z^0 — the carrier of the neutral weak interaction Among the various decay pathways for the Z^0 are pairs of neutrinos (precisely, a neutrino and an antineutrino) Thus, just as water escapes from a leaky bucket faster the more holes there are, so the lifetime of the Z^0 is shorter the more neutrino flavours there are for it to decay into It is the precise measure of this lifetime that has determined the number (N_ν) of light neutrino flavours

The quality of the LEP results may be illustrated by comparison with previous knowledge on N_ν C Hearty *et al*[5] analysed lower-energy data on $e^-e^+ \rightarrow$ a photon (γ) + missing neutral energy If the missing neutral energy is carried by neutrinos, this gives $N_\nu < 5\ 2$ In that same paper, the analysis of W and Z^0 production and decay data at CERN's older proton–antiproton collider gives $N_\nu < 7\ 0$ for the UA1 and UA2 collaborations individually (though this involves further information from other processes and is thus somewhat indirect) The analogous Z^0 production at the Fermilab Tevatron hadron collider was studied by the CDF collaboration[6], this gives a rather good measure of the Z^0 mass but does not significantly limit N_ν

The significant advances have come from the custom-designed electron–positron machines SLC (Stanford Linear Collider) and LEP The SLC uses new concepts in collider technology and is currently accumulating data slowly Their results are limited by the statistics, their original publication (cited in David Miller's News and Views[7]) gave $N_\nu = 3\ 8 \pm 1\ 4$, by the time of the Stanford Lepton–Photon Symposium in August this had improved to $3\ 0 \pm 0\ 9$ and the latest refinement[8] is to $2\ 8 \pm 0\ 6$

LEP is a conventional circular collider that has performed to design specification in a miracle of technological success The four large collaborations have made their first reports on the Z^0 parameters and obtain for $N_\nu = 3\ 42 \pm 0\ 48$ (L3[1]), $3\ 27 \pm 0\ 30$ (ALEPH[2]), $3\ 12 \pm 0\ 42$ (OPAL[3]) and $2\ 4 \pm 0\ 65$ (DELPHI[4]) Thus the odds against even four flavours are drastically lengthened and the three known may be the sum total Moreover, cosmologists defined 'light' as a mass below 1 MeV, the SLC and LEP data extend this by a factor of 40,000 to below 40 GeV

The Z^0 decays to hadrons and charged leptons as well as to neutrinos so the neutrino decays are only a part of the whole, which limits the accuracy of deduction of N_ν The best bet for improving the limits on N_ν seems to be LEP, where the machine works so well that good statistics may be anticipated for $e^+e^- \rightarrow \gamma + Z^0$, where $Z^0 \rightarrow$ neutrinos Thus one will have $e^+e^- \rightarrow \gamma$ + missing neutral energy which is directly proportional to N_ν (the same process as discussed by Hearty *et al* but now at energies where a real Z^0 leads to the neutrinos) without contamination of the other decay channels

P Langacker[9] has taken some of these recent data and studied their implications for the yet undiscovered Higgs boson and top quark masses They do not constrain the former but do constrain $m_t < 210$ GeV (the top quark mass enters into radiative corrections that affect some weak-interaction processes) The weak-interaction angle (Weinberg angle θ_W) is known within errors as a function of m_Z and m_t The precise measure of m_Z thus gives a rather clear linear relation between $\sin^2\theta_W$ and m_t, and it is the errors on $\sin^2\theta_W$ that allow the range of m_t up to 210 GeV

A reduction of these errors, by a more precise measure of m_W/m_Z, will thus lead to a prediction for the mass of the top quark, the missing member of the three families of the standard model And if the SLC and LEP data are to be believed, three is the magic number ☐

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1 Adeva, B *et al Phys Lett* B**231**, 509–518 (1989)
2 Decamp D *et al Phys Lett* B**231**, 519–529 (1989)
3 Akrawy M Z *et al Phys Lett* B**231** 530–538 (1989)
4 Aarnio, P *et al Phys Lett* B**231**, 539–547 (1989)
5 Hearty C *et al Phys Rev* D**39**, 3207 (1989)
6 Abe F *et al Phys Rev Lett* **63** 720–723 (1989)
7 Miller D J *Nature* **340**, 677–678 (1989)
8 Abrams, G S *et al Phys Rev Lett* **63** 2173–2176 (1989)
9 Langacker, P *Phys Rev Lett* **63** 1920–1921 (1989)

PHASE TRANSITIONS

New ideas for the melting pot

Robert W Cahn

MELTING is distinct from other phase transitions in that it is not clear which of several mechanisms is responsible for initiating the process as temperature is raised On page 658 of this issue[1], Tallon joins a debate on what effect each of the possible precursors to melting may have on the extent to which crystals can be superheated

Following my discussion[2] three years ago of the first observations of crystalline superheating above the equilibrium melting point, and the intimate linkage of this phenomenon with the nature of the crystal surface, Maddox[3] described new, strong experimental evidence that melting is indeed initiated at a crystal surface Last year, Fecht and Johnson[4] (with myself acting as chorus[5]) proposed that, under circumstances that permitted superheating at all, there must be an upper limit, thermodynamically determined, to how far superheating can go The central idea was that an 'entropy catastrophe', beyond which the crystalline entropy would exceed that of the liquid phase, would determine this limit A few months later, Lele *et al*[6] proposed an alternative way of estimating the temperature of the upper entropy catastrophe (there is also a lower catastrophe, the Kauzmann temperature, that marks the limit of supercooling of a liquid/glass) It is this same question — the best way to estimate the upper limiting temperature for superheating — that Tallon also addresses

The crucial role of the surface in initiating melting has been underlined in several recent papers The important work by Pluis *et al* to which both Maddox[3] and I[2] drew attention has now been more fully described[7] as the melting point is approached, a layer of disordering gradually develops, its thickness grows very corresponding oligonucleotide probes, and screened brain cDNA libraries by hybridization Wada *et al*[2] and Gregor *et al*[3] have cloned what may well be the frog and chick versions of the same kainate-binding protein (KBP) This protein has a relative molecular mass of about 50,000 (about half that of the kainate receptor channel identified by Hollmann *et al*) and, again, the hydrophobicity plot typical of the ligand-gated-channel gene family The KBP exhibits about 25 per cent amino acid similarity to the last (carboxy) half of the kainate receptor channel cloned by Hollmann *et al* , but the first 350 amino acids of the kainate receptor channel are entirely absent from the KBP The KBP binds kainate, as it should, but does not function as a channel when expressed in oocytes

In summary, then, Hollmann *et al* have cloned at least one subunit of a functional glutamate receptor channel, the other two laboratories have cloned a related protein that binds glutamate (kainate) but whose function is at present unknown What could this KBP be? One possibility is that it is a subunit of a neuronal kainate receptor and that the channel will only function when other subunits are present Because the chick KBP is remarkably abundant in cerebellum and is only present on Bergmann glial cells there, other possibilities are likely The KBP could, for example, be a component of an uptake system or part of a glutamate receptor involved in a signalling that does not use an ion channel Glia have recently been shown to transmit signals to one another in response to glutamate[4], and this signalling system, which involves changes in intracellular calcium concentration, responds selectively to kainate Whatever the KBP is, it belongs to a previously undefined family, and because of its abundance in glia rather than neurons, it must play a role in some as yet unknown aspect of brain function

For neurobiologists, the cloning of a glutamate receptor channel is an exciting event To understand why, one must appreciate the role of glutamate receptor channels in brain function First, glutamate is the main excitatory neurotransmitter in the brain, and every neuron — as far as we know — has ion channels with receptors for glutamate The glutamate receptors are thought to form a complex family because they fall into a rich variety of pharmacological types, and, if past experience is any guide, the gene families will be even richer than the current pharmacology indicates Second, at least one pharmacologically defined type of glutamate receptor, the one named after the glutamate analogue NMDA (*N*-methyl-D-aspartate) that best activates it, is especially interesting The NMDA receptor is a site of action of the street drug, 'angel dust', is responsible for excitotoxic nerve cell death and has a key part in long-term potentiation, the phenomenon thought to underlie some forms of memory Furthermore NMDA receptors have been implicated in certain types of seizure and various neurodegenerative diseases, and in the reorganization of neuronal connections that depends on experience Heinemann's laboratory, from which the paper by Hollmann *et al* comes, has already started finding other family members related to their kainate receptor channel, and it is expected that an entirely new family of receptors, one that may include the NMDA receptor, will be identified through homology screening

Finally, an interesting sociological note Although the three groups that came first in the race to clone a glutamate receptor worked independently (and competitively), the research of two of them was carried out in different laboratories at the Salk Institute, and one of the co-authors in the third group was in yet another laboratory at the Salk Something about southern California must be good for research on excitatory amino acids ☐

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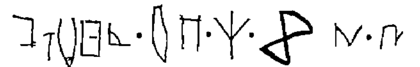
1 Hollmann M O Shea-Greenfield, A Rogers S W & Heinemann, S *Nature* **342**, 643–648 (1989)
2 Wada, K *et al* *Nature* **342**, 684–689 (1989)
3 Gregor P Mano, I Maoz I , McKeown M & Teichberg V I *Nature* **342** 689–692 (1989)
4 Cornell-Bell A , Finkbeiner, S M , Cooper M S & Smith, S J *Science* (in the press)

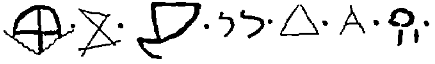
100 years ago

EARLY EGYPTIAN CIVILIZATION

THOUGH debarred from the richest districts of Egypt — owing to national jealousies — I have been fortunate enough to discover two small towns which have revealed the works of the Middle and New Kingdoms with chronological exactness Beside the Egyptian interest of these places, they are of prime importance for Mediterranean history, having been colonies of foreign workmen The most novel discovery of



Inscription on wood Signs incised in pottery



Signs incised on pottery of the twelfth dynasty

all is the presence of apparently alphabetic signs in use in both towns (figure) at about 2500 B C and 1300 B C The apparent connection of these signs with some of the mason's marks suggests that they may have been adopted by the foreign workmen from their Egyptian fellow-labourers, and the very lack of literary education among such men would lead to their forming alphabets of their own

From *Nature* **XLI**, 109–111 (1889)

BIOGEOCHEMISTRY

Effects of increased ultraviolet

T E Graedel

THE discovery of the Antarctic ozone hole has sparked tremendous discussion about the implications of increased ultraviolet radiation on geochemical and biochemical processes Because many of the atmospheric and marine chemical cycles strongly interact with those of the biota, changes in any of the systems imply changes in all These interlocking concerns formed the basis for a recent workshop* which brought together atmospheric chemists and physicists, marine and freshwater chemists, biochemists and biologists

The effects of changing ultraviolet fluxes can be studied by examining each term of what one might call the photochemical impact equation For a specific molecule i, the impact I_i can be written as

$$I_i = \int_{\lambda_{min}}^{\lambda_{max}} J(\lambda)\, \sigma_i(\lambda)\, \phi_i(\lambda) \mathrm{d}\lambda$$

in which $J(\lambda)$ is the incident flux, $\sigma_i(\lambda)$ is the absorption cross section and $\phi_i(\lambda)$ is the quantum efficiency (that is, the fraction of absorbed photons that cause the process under study, as opposed to having their energy dissipated in some other way) The integration limits are the atmospheric cut-off at about 290 nm at the short wavelength end, and the longest wavelength capable of producing an effect (generally in the range 350–400 nm) at the upper end

In the case of absorption by agglomerated particles — poorly characterized dissolved species or organisms, for which molecule-by-molecule approaches are inappropriate — the absorption cross-section and quantum yield terms are replaced by $A_i(\lambda)$ This 'action spectrum' is an experimentally determined measure of the effects of the interaction of light at a particular wavelength with a specific constituent A quantitative assessment of the effects of changing ozone concentrations requires a relatively complete understanding of each term in these equations, speakers at the workshop presented results related to each term

Changes in atmospheric ozone are now being monitored widely It does not follow, however, that even $J(\lambda)$, the present flux of solar radiation to the surface, is known accurately, because clouds play such a large role in the radiation balance The distribution and physical properties of clouds are not well characterized, particularly over the oceans and

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**Effects of Solar Ultraviolet Radiation on Biogeochemical Dynamics in Aquatic Environments* Woods Hole, Massachusetts 23–25 October, 1989 the poles Calculations by S Madronich (National Center for Atmospheric Research, Boulder) predict that for mid- and low-latitudes, fluxes of biologically active UVB (290–320 nm) at the surface will change by −5 to +10 per cent by the year 2060, depending both on the model and the assumed course of ozone depletion For southern polar regions, large increases in springtime ultraviolet are expected (as much as a factor of ten in the year 2060) But because the average UVB irradiance is much higher at equatorial latitudes than at the poles, a percentage increase in UVB irradiance in the tropics corresponds to many more additional photons than the same increase at high latitudes

Increases in solar ultraviolet radiation seem certain to produce substantial changes in the composition of the atmosphere as a consequence of the absorption of the radiation by gaseous molecules Calculations by A M Thompson (NASA/Goddard Space Flight Center) indicate that the oxidizing power of the lower atmosphere will be much increased and that the atmospheric content of many trace gases scavenged by oxidizers will decrease Cloud effects pose large uncertainties, however, because soluble gas-phase species can interact with clouds (and aid in their formation) in ways not well understood, and because most atmospheric chemical models have yet to treat cloud effects

P Crutzen (Max-Planck-Institute for Chemistry) reported recent calculations in which cloud chemistry was added to a global two-dimensional model Although clouds occupy only about 15 per cent of the volume of the lower troposphere and liquid water occupies only 10^{-7} to 10^{-6} of a cloud's volume, the inclusion of clouds in the model greatly decreased the calculated concentrations of ozone, hydroxyl radical and nitrogen oxides, thereby affecting the budgets of many other trace gases (P Crutzen and J Lelieveld *Nature*, in the press)

Within condensed phases, the results of increased ultraviolet are also likely to be dramatic A few years ago, there was little evidence for the existence of reactive chemical transients in environmental waters Recently, however, several studies using carefully designed trapping-agent molecules have established the presence of such species as excited states, singlet molecular oxygen, solvated electrons, hydroxyl radicals, superoxide radicals, carbon-centred radicals and hydrogen peroxide All are generated by solar ultraviolet radiation Hydrogen peroxide is the longest lived of these transients and its widespread occurrence in the upper ocean and freshwaters provides an important marker of the influence of solar ultraviolet radiation in aquatic environments

The biological effects, on both plants and animals, of increased ultraviolet radiation can be assessed by species only if each $A_\lambda(\lambda)$, the appropriate species action spectrum, has been determined Useable action spectra are, however, rare T Coohill (Western Kentucky University) highlighted some of the problems uncertainty in the spectral purity of the incident radiation, difficult dosimetry determinations, the extent of biological repair before assay, the physical state of the target molecules, and response *in vivo* versus response *in vitro* Hence, assessments of the effects of changing ultraviolet radiation are, at present, much more likely to be qualitative than quantitative

In one of the workshop's more provocative presentations, F M M Morel (Massachusetts Institute of Technology) argued that one could logically defend any of the following positions Increased ultraviolet radiation will promote a dramatic increase in new primary biotic productivity, practically destroy biotic productivity, or have no discernible effect on biotic productivity His claim can be made because the quantitative information to determine the dominant effects is generally unavailable and because different factors seem likely to dominate at different places, times and seasons

Morel's first position was supported by several participants who pointed out that the bioproductivity of the oceans often seems to be limited by the availability of trace nutrients such as iron, copper and manganese Photochemical reactions involving bound forms of these elements increase their availability Hence, regions of the oceans that are now nutrient-limited may benefit from increased ultraviolet radiation

The converse position — that the increased damage to DNA will overwhelm any benefits in nutrient supply — was supported by several reports that UVB radiation markedly reduces growth in some species of marine phytoplankton The biological impact is related to the number and efficiency of DNA repair systems in organisms Field studies from Palmer Station, Antarctica (D Karentz, University of California, San Francisco) demonstrate that there are enormous interspecies differences in sensitivity to ultraviolet

Could increased ultraviolet fluxes have only little effect on biotic productivity? T Mill (SRI International) argued that concentrations of marine and freshwater reactive chemical transients are too low and their lifetimes too short to have major adverse effects on the biota It is also worth considering that marine phytoplankton already cope with reactive photoproducts and trace-element depletion ☐

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DAEDALUS

Air of longevity

ONE of the appealing hazards of air travel is the ease with which you can get drunk The reduced pressure and oxygen content of the cabin slows the metabolic elimination of alcohol, giving two drinks almost the effect of three Daedalus therefore began to design a low-pressure pub Drinkers would have to enter by an air-lock, but could then get drunk more cheaply, and stay that way longer Conversely, increased pressure should speed up alcohol metabolism A high-pressure bedroom, for example, might enable the alcoholic to burn off his entire hangover overnight, and greet the morning after with a clear head

Daedalus is now extending this whole principle He points out that if fed on restricted diets, nearly all animals mature more slowly and live longer than usual Every creature studied seems to step through its whole 'life programme', from growth and sexual maturity to senescence and death, by the ticking of its metabolic clock Animals raised in low pressures should therefore have longer, slower biological careers. If the principle works with man as well, then by spending your whole life in a cruising aircraft you might live to be a hundred Or perhaps it would only seem like that

Low pressure may even space out the minor milestones of life The irregular menstrual cycle of many airline stewardesses has been attributed to their chaotic shuttling between time zones Daedalus suspects instead that they 'age' more slowly in the air, and get out of step with ground-level time

So DREADCO's physiologists are setting up a variable-metabolism unit To avoid the problems of pressurized construction, Daedalus contemplated slowing the oxygen metabolism of the inmates by introducing a little carbon monoxide into the air of the building But wiser counsels prevailed, and instead the oxygen concentration of its air will be varied directly Daedalus expects that his under-oxygenated volunteers will lose appetite and need only two meals a day To study ageing itself, he is appealing for middle-aged identical twins, one to live in the low-oxygen laboratory and one outside while microscopic examination compares the rate at which grey hairs appear on their scalps

The effects of oxygen-enriched air should also be interesting Metabolism may speed up sharply, so that obese volunteers are swiftly 'burnt down' to normal weight Animals may grow faster an oxygenated poultry house, for example, might hurry chickens to earlier maturity It would be hard to extend such atmospheric modifications to free-range life, whether in chickens or man, though an oxygen boost on the air conditioning might be welcome in many crisis-prone offices David Jones

Pneumologists breathe easy

SIR—I have read with interest the article "Model Lung Breathes True"[1] and the quoted paper by two physicists, Bonsignori and Salvini, *Il Nuovo Cimento*[2], which is said to "contain an intriguing calculation of gas transport in the human lung of a kind that would normally have been found in a physiological journal". In fact, the results of similar calculations belong to standard physiological textbooks[3]. In my opinion, the paper of Bonsignori and Salvini is only relevant to the respiratory physiology of the early 1970s.

Whatever the model (in physiology as in physics), comparison with experimental observations is the crucial test. What Maddox has written[1], that "the outcome of the calculations is much what physiologists would expect", is not true. The authors simulated a simple experiment, called single breath nitrogen washout, consisting of an inspiration of oxygen followed by an exhalation. Most of the expired-N_2 concentration profile, the so-called alveolar plateau, is not flat, as would be expected if the lung is a perfect mixing chamber. (The slope of the alveolar plateau is one of the standard indices in pneumology and is explained to medical students in elementary textbooks.)

The model of Bonsignori and Salvini simulates a flat alveolar plateau and the authors say that "in experiments, the single breath washout curves have rather flat alveolar plateaus, indicating uniform mixing of the alveolar and inspired gases"[2]. It is well known, since the experiments of Krogh and Lindhard[4] at the beginning of the century, that this is not the case. In other words, the lung model of Bonsignori and Salvini does not breath true.

Maddox also quotes Bonsignori and Salvini as claiming to have improved on some of the approximations to which previous calculations have been subject. These improvements consist of 1, using a better representation of the airway cross-section, 2, using a Taylor–Aris effective diffusion, and 3, discussing more appropriate boundary conditions. In a review on the subject[5], several references (probably not known by Bonsignori and Salvini) are given where all these points have been studied. None of these have significantly improved agreement between the model simulations and the experimental observations.

As Maddox says, the physiological data are striking, the total cross-section of airways increasing from a few square centimetres in the trachea to more than a square metre at the last generations of the bronchial tree. A major consequence of the lung geometry is that when we inspire at constant flow, the concentration profile of the inspired gases remains quasi-stationary, the convection flow of inspired gases towards the lung periphery being balanced by the diffusion flow of the residual (or alveolar) gases in the opposite direction[5]. This is the so-called diffusion front, equivalent to the stationary diffusion front in combustion science, when the combustion takes place in a tube with the appropriate convection velocity of gases.

A major development of models of gas mixing in the lung (not quoted by Bonsignori and Salvini) was the realization[5] in the 1970s that one of the most fragile assumptions in previous work consists of using the symmetrical Weibel model of the lung[6], which dates back to 1963. Not surprisingly, the first detailed asymmetrical model of the periphery of the human lung was recently published by Weibel's group[7]. Unexpected results appeared when a diffusion equation was written in an asymmetrical structure of ducts as a consequence of Fick's law of diffusion and matter balance in a branching tube: the flow of any gas in such a structure generates an inhomogeneous concentration distribution, even though the structure moves isotropically and homogeneously. This occurs in zones of the model that are subtended by branch points where the Péclet number (giving the relative contribution of convective to diffusive transport) is approximately unity. In the human lung during normal breathing, this corresponds to branch points subtending zones of the lung a few millimetres apart.

Simulated single-breath washout using these new models gives much better agreement with experimental observations than previous models[5]. This finding is of interest in that the single-breath washout, particularly when performed with tracer gases of different diffusivities such as He and SF_6, gives information on the structure of the very peripheral zones of the lung which are not accessible to traditional tests in pneumology nor even to modern imaging techniques such as positron emission tomography. To my knowledge, the description of gas transport in an asymmetrical lung model has no counterpart in physics or engineering, although it may have applications in the study of gas transport in porous media, or O_2 and CO_2 transport in some insect-built galleries, where the Péclet number may approach unity. It may also apply to the study of O_2 transport between capillaries and cells.

Physiology (which, in a way, is the physics of living beings) and physics were closely associated until the last century, and several discoveries in both fields were made by the same scientist: Boyle, Torricelli, Pascal, Hooke, Lavoisier, Laplace and Lagrange have contributed to the early development of respiratory physiology, and both Poiseuille and Fick were physicians. The bridge is much narrower today and the paper by Bonsignori and Salvini in *Il Nuovo Cimento* has the merit, as Maddox says, of establishing a new link. It also points out the necessity for the physicists who approach physiology to learn at least its elementary basis, if they want to avoid the trap of rediscovering the wheel. Bonsignori and Salvini promise a new paper where they will pay particular attention to the question of the boundary conditions in the model. If they do not in the meantime read the published literature, respiratory physiologists need not hold their breath awaiting future issues of *Il Nuovo Cimento*.

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1. Maddox, J. *Nature* **341**, 381 (1989).
2. Bonsignori, F. & Salvani, M. *Il nov. Cim.* **11D**, 1247–1263 (1989).
3. Engel, L. A. in *Handbook of Physiology* (American Physiological Society, Bethesda, Maryland, 1986).
4. Krogh, A. & Lindhard, J. *J. Physiol. Lond.* **51**, 59–90 (1917).
5. Paiva, M. & Engel, L. A. *Physiol. Rev.* **32**, 750–796 (1987).
6. Weibel, E. R. *Morphometry of the Human Lung* (Springer, Berlin, 1963).
7. Haefeli-Bleuer, B. & Weibel, E. R. *Anat. Rec.* **220**, 401–414 (1988).

PCR origins

SIR—We noted with interest the short note, entitled "DNA amplification" (*Nature* **341**, 570, 1989) which suggests that scientists who use PCR "dust off" the *Journal of Molecular Biology* from 1971, and read the paper by "H. Gobind Khorana and colleagues". Although the title and text of your note might suggest otherwise, the "lines of thought" and reference to experiments "in progress" in this 1971 article did not present a method that could be used to perform *in vitro* DNA amplification. The scientific community had to wait for such a method until, 14 years later, Kary Mullis invented the polymerase chain reaction (PCR).

Your readers might be interested to know that the articles written by Kary Mullis, Henry Erlich and their co-workers at Cetus describing the PCR method and its application have been cited in well over 1,000 publications since 1985. None of these publications cite the 1971 article to which you draw attention. Cetus is confident that Kary Mullis will continue to receive the recognition he deserves.

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Similarity in membrane proteins

SIR—Adams and Pollard[1] recently discovered that the nonfilamentous myosins IA and IB of *Acanthamoeba* can bind to membranes, a finding with important implications concerning the role of these proteins in cell motility We would like to draw attention to a 50-amino-acid domain in the sequence of the myosin-IB isozyme[2] that is shared with a diverse family of cytoplasmic proteins, many of which are associated with the plasma membrane (see figure) This domain, called SH3 or the A-box, was first described as a region of sequence similarity between the non-receptor protein-tyrosine kinases, the 148K phospholipase C (PLC), and the v-*crk* oncogene of the avian sarcoma virus CT10 (refs 3 and 4) It was subsequently found in the α-chain of the non-erythroid spectrin homologue, fodrin[5]

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Myosin-1B  (983)   ALYDFAAENPDE-----LTFNEGAVVTVI-NKSNPDWWEGEL----NGQA-GVFPASYVELI-PR   ref 2
Fodrin-α   (974)   ALYDyqeksPrE-----vTmkkGdiITIl-NstNkDWWkvEv----Ndrq-GfvPAaYVkkl-dp       5
PLC148     (798)   ALfDykAqreDE-----LTFtksAiiqnv-eKqeggWWrGdyh---hkkq-lwFPsnYVEemv-s       3
v-Crk      (375)   ALfDFkgnddgd-----LpFkkGdilkir-dKpeeqWWnaEdm---dGkR-GmiPvpYVEkcrPs       4
c-Fgr      (84)    ALYDyeArteDd-----LTFtkGekfhil-NntegDWWEarsls--sGkt-GciPsnYVapv-ds       9
Lsk        (67)    ALhsyepshdgd-----LgFekGeqlril-eqS-geWWkaqslt--tGQe-GfiPfnfVaka-ns      10
c-Yes      (97)    ALYDyeArtted-----LsFkkGerfqiI-NntegDWWEarsia--tGkn-GyiPsnYVapa-ds      11
Hck        (62)    ALYDyeAihred-----LsFqkGdqmvVl-eea-geWWkarsla--tke--GyiPsnYVarv-ns      12
c-Src      (88)    ALYDyesrtetd-----LsFkkGerlqiv-NntegDWWlahslt--tGQt-GyiPsnYVaps-ds      13
c-Abl      (68)    ALYDFvAsgdnt-----LsitkGeklrVlgynhNgeWcEaqtk---NGQ--GwvPsnYitpv-ns      14
fus1       (443)   viqDyeprltDE-----irislGekvkil-athtdgWclvqkc---NtQk-GsihvSvddkryln     6,7
cdc25      (64)    AaYDFnypikkdsssqlLsvqqGetiyil-NKnssgWWdGlviddsNGkvrGwFPqnfgrplrds      15
Consensus          ALYDY------D-----Φ-Φ--GD-Φ-ΦΦ-------WW-------------GΦΦP--YΦ------
                     F F      E           E                                 F
```

Upper-case letters denote identity with the myosin-IB sequence The amino-acid number of the first residue of each sequence is given in parentheses ϕ signifies hydrophobic residues The PIR database was searched using the programs Prosearch (J Collins and A Coulson, University of Edinburgh) on an AMT DAP, and AMPS (G Barton and M J E S) on a VAX 8700, the Genepro program (Riverside Scientific, Seattle) was used to search the EMBL database

Our database searches have also revealed SH3 domains in cdc25 and fus1, two proteins of another single-celled eukaryote, *Saccharomyces cerevisiae* The fus1 protein on the cell membrane is essential for cell fusion during mating[6,7], cdc25 is a regulator of the membrane associated adenylyl cyclase The SH3 motif is, therefore, shared by membrane-associated proteins of both single-celled and higher eukaryotes This conservation indicates a basic function common to all eukaryotes but this function is still a mystery Although SH3 is found in many proteins at the cell periphery, it is not universal, it is absent from erythroid spectrin for instance

Adams and Pollard[1] mapped the membrane-binding region of myosin-IA to a 100K N-terminal chymotryptic fragment, but the binding region of myosin-IB has not yet been localized Intriguingly, the SH3 domain of the IB protein is close to the protein's ATP-independent actin-binding site[8] It will be interesting to see whether the SH3 domains of myosin-IB and other proteins play a role in binding either directly to membranes or to the submembrane cytoskeleton

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1 Adams R & Pollard, T *Nature* **340**, 565–568 (1989)
2 Jung G Korn E & Hammer J *Proc natn Acad Sci U S A* **84** 6720–6724 (1987)
3 Stahl M Ferenz, C Kelleher, K , Kriz, R & Knopf J *Nature* **332** 269–272 (1988)
4 Mayer B Hamaguchi, M & Hanafusa, H *Nature* **332** 272–274 (1988)
5 Wasenius V *et al J cell Biol* **108**, 79–93 (1989)
6 Truehart, J , Boeke J & Fink G *Molec cell Biol* **7** 2316–2328 (1987)
7 McCaffrey G , Clay F Kelsay, K & Sprague, G *Molec cell Biol* **7**, 2680–2690 (1987)
8 Lynch, T *et al J biol Chem* **261**, 17156 (1986)
9 Katamine, S *et al Molec cell Biol* **8**, 259–266 (1988)
10 Marth, J Pect, R , Krebs E & Perlmutter R *Cell* **43** 393–404 (1985)
11 Sukegawa, J *et al Molec cell Biol* **7**, 41–47 (1987)
12 Klemsz M McKercher S & Maki R *Nucleic Acids Res* **15** 9600 (1987)
13 Takeya T & Hanafusa H *Cell* **32**, 881–890 (1983)
14 Shtivelman E , Lifshitz B , Gale, R Roe, B & Canaani E *Cell* **47**, 277–284 (1986)
15 Broek D *et al Cell* **48** 789–799 (1987)

Retrocitation

SIR—In my News and Views article "Reverse Transcriptases Retrons in Bacteria" (ref 1) I stated "no such reverse transcriptase seemed to exist in bacteria" M Beljanski has since called my attention to his publications on reverse transcriptase in bacteria[2–5] This work was confirmed in several Soviet publications[6–9] It will be of interest to see if these activities are related to retrons

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1 Temin, H M *Nature* **339** 254–255 (1989)
2 Beljanski, M *C r hebd Seanc Acad Sci Paris* D**274**, 2801–2804 (1972)
3 Beljanski M *C r hebd Seanc Acad Sci Paris* D**276** 1625–1628 (1973)
4 Beljanski, M & Beljanski M *Biochem Genet* **17**, 163–180 (1974)
5 Beljanski M , Bourgarel, P & Beljanski, M S *C r hebd Seanc Acad Sci , Paris* D**286**, 1825–1828 (1978)
6 Romashchenko A G *et al Dokl Akad Nauk SSSR* **233**, 734–737 (1977)
7 Lushnikova T P *et al Molec Biol* **12** 1163–1171 (1978)
8 Vorob'eva N V *et al Molec Biol* **16**, 977–983 (1982)
9 Grabkina O A *et al Molec Biol* **17** 830–838 (1983)

What are males good for?

SIR—Successful rearing of young in most birds requires the care of two adults Such parental pairs are almost always a male and a female, but the discovery of female/female pairings in one tern, one goose and several gull species, discussed by Jared Diamond[1], demonstrates that other parental pairing combinations are possible Female/female pairs are, nevertheless, rare even in these few species[2–6] Determining the reasons why they are so uncommon may help to formulate answers for the old question 'What are males good for?'

The most obvious benefit to females of pairing with a male — access to a source of sperm — is probably not of primary importance, as females paired together still copulate with unmated and mated males[7] Female/female pairs, therefore, produce fertile eggs, although their fertility rates may be less than those of male/female pairs[4–6]

Perhaps a more important advantage provided by males is that of a territory Female birds are generally smaller than males, and may, therefore, be at a disadvantage when competing against a male for a territory In all the colonies where I have found female/female pairs or the unusually large (5–7 egg) clutches that result from them[2,8] (eight ring-billed gull colonies, four California gull colonies and a Caspian tern colony), space for more nesting pairs was available on the nesting islands In western gulls, female/female pairs were found in one colony where there was little competition for breeding space, but no such pairs were found in another colony where breeding space was limited[9]

Probably the most important advantage a female derives from pairing with a male is the opportunity to raise only her own young (nest parasitism aside) By contrast, each female in a homosexual pair will contribute, on average, only half the young An unrelated pair of females must, therefore, produce twice the offspring of a heterosexual pair before the reproductive output of each homosexually paired female equals that of a

1 Diamond J M *Nature* **340** 101 (1989)
2 Conover M R *Condor* **86** 467–471 (1984)
3 Conover, M R *Wilson Bull* **96** 249–267 (1984)
4 Hunt, G L Jr & Hunt M W *Science* **196**, 1466–1467 (1977)
5 Kovacs K M & Ryder J P *Auk* **100**, 658–669 (1983)
6 Quinn T W *et al Auk* **106**, 177–184 (1989)
7 Fitch M A & Shugart, G W *Am Nat* **124**, 116–126 (1984)
8 Conover M R *Condor* **85** 346–349 (1983)
9 Pierotti, R *Auk* **98**, 532–549 (1981)
10 Lagrenade, M & Mousseau P *Auk* **100** 658–669 (1983)
11 Hunt G L Jr *et al Auk* **97** 473–479 (1980)
12 Conover M R & Hunt G L Jr *Condor* **86**, 472–476 (1984)
13 Conover M R & Hunt, G L Jr *Wilson Bull* **96**, 619–625 (1984)

of the aborted zygotes This must also be true in species where it has been shown (by experimentally destroying competing embryos) that aborting embryos are not intrinsically inviable[14] Such evidence also rules out maternal choice among embryos, or sibling competition, if based on the genotypes of the embryos In many species, however, nonrandom abortion is difficult to demonstrate, and most species have some random abortion We cannot therefore exclude a contribution of mutational load to low female fertility

It is certainly worth considering the additional possible causes mentioned by Bawa *et al* for the patterns of abortion and low ovule fertility seen in some plants (parent–offspring conflict, providing excess ovules so that maternal choice can occur, and pollen competition) These hypotheses are difficult to test, and are at present rather controversial, and I did not have space to mention them in my brief discussion of the question The first is closely related to the high cost of fruit and seed maturation, discussed in my News and Views in terms of theories of resource allocation which show that this cost can lead to the evolution of low female fertility, even though potentially viable progeny are lost Pollen competition does not seem a likely explanation for species with regularly low seed to ovule ratios, because some pollen sources should yield seeds from every ovule, which does not appear to be possible in such plants[15]

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WIENS *ET AL* REPLY—Our letter to *Nature*[2] reported extensive (97 5 per cent) genetically mediated developmental failure of ovules and consequent loss of reproductive capacity in *Dedeckera eurekensis* (Polygonaceae), a rare, palaeoendemic, monotypic genus from the Mojave desert of California

Bawa *et al* question both the uniqueness and interpretation of our results They indicate that mimosoid legumes have exceptionally low fruit/flower ratios (per cent fruit set), but relatively high seed/ovule ratios (per cent seed set) giving these plants overall seed set similar to *Dedeckera* (2–3 per cent) We have confirmed these results in African *Acacia* and North American *Prosopis* Apocynaceae and Asclepiadaceae behave similarly, as do many woody groups, and plants reproducing vegetatively These reproductive systems, however, are not comparable to that of *Dedeckera*

In mimosoid legumes low fruit set is genetically programmed by the maternal genome The great majority of ovules are probably unfertilized, or if fertilized, probably abort before the activation of the zygotic genome at differentiation, or their maturation is physiologically inhibited by earlier developing fruits Such ovules never enter the potential seed pool

Maternal fitness, however, is determined by the absolute number of fully viable seeds produced In spite of the low fruit set per inflorescence, a mimosoid may produce hundreds or possibly thousands of inflorescences with one to several fruits and thousands of seeds

In *Dedeckera* more than 95 per cent of the ovules are fertilized (flowers are uniovulate) and develop to various stages The few filled seeds that mature occur randomly in the inflorescence, any ovule can potentially enter the seed pool The 97 5 per cent abortion rate among *Dedeckera* ovules, however, represents only a portion of the reproductive loss Large, vigorous plants of *Dedeckera* may produce about 64,500 flowers (and ovules) Of the 2 5 per cent of filled seeds only 3 5 per cent germinate spontaneously, and only 11 per cent are without post-developmental abnormalities Most *Dedeckera* plants probably produce less than 50 fully viable seeds annually Some plants are apparently totally sterile Seed inviability of this magnitude seems unprecedented among any evolutionary successful species

Severely reduced seed sets (inbreeding depression) are often encountered when typically outcrossing plants are selfed Such results are of little interest The similarly low seed sets obtained in *Dedeckera* from both outcrossing and inbreeding are precisely what make it so unusual The genetic load in *Dedeckera* is perhaps so high that rare, recombinant gametes produced upon selfing may have as great a probability of producing a viable embryo/endosperm as outcrossed progeny A number of phylogenetically and geographically rare plants (monotypic families) seem to have reproductive capacities similar to *Dedeckera* These endangered plants may also exhibit unusual genetic phenomena not heretofore encountered They require urgent study

The failure of ovular development in general is attributable to several causes Nonrandom abortion (maternally controlled) is exceedingly common and likely under fixed genetic control of the maternal genome[16] Random ovule abortion is primarily attributable to genetic load and developmental selection[16,17]

Our previous letter on *Dedeckera* is essentially concerned with reduction of fitness, and presents a difficult evolutionary conundrum If the loss of fecundity resulted in continued population decline, why would selection not reverse the trend? We proposed a heterosis (balanced-load) model that allowed for the survival of unique, highly heterozygous genotypes in an environment stressed by increasing aridity Such genotypes should suffer drastically reduced fecundity from genetic load Selection, however, must firs ensure individual survivorship, thei fecundity This could ultimately lead t extinction

How can such a situation be reconcile with sibling rivalry/parent–offspring conflic hypotheses? How can they explain th high loss of seed viability, germinability and the presence of extensive postger mination developmental abnormalities all of which follow the release of seed from the maternal plant? Likewise, ho can they account for embryo death similar to those known to be controlled b developmentally lethal genes[18]

These highly anthropomorphic, socio biological hypotheses are best not applie to plants We suggest they cannot b tested critically, and have no mechanis to explain their operation The causal mechanistic genetic based hypotheses although not without difficulties, ar founded on established phenomena an Occam's razor dictates their acceptance

Since publication of our letter, we hav carried out a more extensive crossing pro gramme in which *Dedeckera* showed tha of 157 self-pollinated flowers (uniovulate one filled seed was obtained, and of 19 cross-pollinated flowers, 23 filled seed (12 0 per cent) were obtained (N = plants)

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1 Charlesworth D *Nature* **338** 21–22 (1989)
2 Wiens D , Nickrent, N L Davern, C I Calvin, C L Vivrette, N J *Nature* **338**, 65–67 (1989)
3 Bawa, K S & Webb, C J *Am J Bot* **71**, 736 (1984)
4 Charnov, E L *Proc natn Acad Sci U S A* **76** 2480-2484 (1979)
5 Lee T D *Am Nat* **123**, 427–432 (1984)
6 Uma Shaanker R Ganeshaiah K N & Bawa K S *A Rev Ecol Syst* **12**, 253–279 (1981)
7 Bawa, K S & Buckley D P *Miss Bot Gard Monog Syst Bot* **29** 243–262 (1989)
8 Bawa, K S *Evolution* **28** 85–92 (1974)
9 Sutherland, S & Delph L *Ecology* **65**, 1093 (1984)
10 Ganashaiah, K N & Uma Shaanker, R *Oecologia* **77** 135–140 (1988)
11 Stephenson, A G *A Rev Ecol Syst* **12** 253–27 (1981)
12 Stephenson A G & Winsor J A *Evolution* **40**, 453–45 (1986)
13 Bookman, S S *Evolution* **38** 1337–1349 (1984)
14 Caspar, B B *Evolution* **31**, 495–511 (1977)
15 Willson M F & Price P W *Evolution* **31** 495–51 (1977)
16 Wiens D *et al* *Oecologia* **71**, 501–509 (1987)
17 Klekowski E J *Mutation Developmental Selection, an Plant Evolution* (Columbia University Press, New York 1988)
18 Meinke, D *et al* in *Plant Genetics* (ed Freeling, M 129–146 (Liss, New York, 1985)

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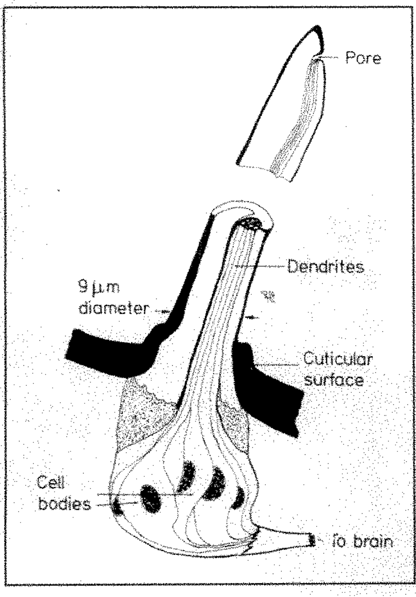

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Anatomy of a conflict

Shepard Krech III

Archaeological Perspectives on the Battle of the Little Bighorn. By Douglas D. Scott, Richard A. Fox, Jr, Melissa A. Connor and Dick Harmon. *University of Oklahama Press: 1989. Pp. 309. Hbk $24.95.*

WHAT more could possibly be said about the Battle of the Little Bighorn that has not been? An event that electrified the country the instant it happened, it continues to excite the imagination. Biographies have been written on the principals involved on both sides — George Armstrong Custer, Crazy Horse and others — as have numerous detailed accounts, from both Indian and white perspectives, of the battle itself and the historical circumstances leading up to and following this epic confrontation, on 25 June 1876, between the US Seventh Cavalry and the Sioux and Cheyenne Indians.

Illuminating the past — H. Charles McBarron's painting of Custer's last stand.

Peter Newark's Western Americana

There are no lingering significant controversies in the interpretation of this historical record. But because there are different perspectives, and because the production and re-production of history is invariably a cultural, presentist exercise, conflicts persist over the interpretation of details in the voluminous historical archive, which probably will occupy historians well into the future. Nevertheless, when one reads on a book jacket that assembled therein is "the most compelling and convincing evidence thus far of what happened on that day in southeast Montana 113 years ago", one can be forgiven for suspecting a publisher's marketing department of hyperbole.

Indeed, the aims of the authors of this book are far more modest, even though at times, it seems, they also are caught up in Little Bighorn hype when they are not actively proselytizing for the revisionist potential of historical archaeology. Grasping the opportunity presented by a grass fire that removed vegetation on the Custer Battlefield National Monument, the authors (of the four, one is a military archaeologist, another an expert on firearms) undertook a comprehensive programme in historical archaeology to determine where 28 soldiers killed in a cul de sac were interred; whether marble battlefield markers actually were placed over human remains; what the bones of troops reveal about diet, trauma and death; how combatants moved about the battleground; and what firearms were used.

The results of archaeological investigations — metal-detector surveys, shovel and backhoe excavations — are mixed. The authors failed to discover the remains of the last 28 soldiers, but they think they now know, by the use of geomorphology and archaeology techniques, where the troopers cannot be and where they are likely to be found. As for the markers, some are improperly placed, not surprisingly because the dead were 'buried' three or four times: the day after the débâcle, the bodies were covered; in 1877 and 1879, exposed remains were reinterred; and in 1881, disinterment was followed by reburial in a mass grave. Nine years later, marble markers were placed on the battlefield, and the following year a map was prepared. Little wonder there are discrepancies. The bones show ample signs of trauma after death, to which all historians concur and about which, for cultural context, Indian sources are most revealing.

The distributions of cartridge-case, bullet and other artefactual materials on the battleground make interesting figures, despite predictable (and wooden) assessments — "the number of bullets impacted in the defence perimeter strongly suggests that the incoming fire must have been heavy" (p. 140). Although plotting distributions leads to a "further clarification" (p. 138) of the positions of the combatants or "correlates well with the historically known combatant positions" (p. 134), it unfortunately adds little to the known historical record.

The most interesting results concern the identification and analysis, on the basis of cartridge cases and bullets, of types and quantities of firearms used by Indians and whites. More than 40 types of guns were used in the battle, and the distribution of types — even of individual weapons — can be derived from signatures that guns leave on bullets and cartridge cases. Even here, however, the archaeological record in part corroborates extant archival materials: a list of guns surrendered by the Sioux and Cheyenne in 1877.

Despite their evident enthusiasm for historical archaeology, the authors wisely state that they do not believe it represents "the last work" (p. 11) in the study of the Little Bighorn. Indeed, apart from often being able to corroborate the known historical record, the conclusions that one can draw from this work are limited Similarly, where the historical record is in dispute, the illuminations provided by historical archaeology are few, with one exception: bullet and cartridge-case distributions support Indian, not white, accounts of the battle. ☐

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Keeping them up in the air

Thomas A. McMahon

Bird Flight Performance: A Practical Calculation Manual. By C. J. Pennycuick. *Clarendon: 1989. Pp.153. £25, $49.95.*

On the wing — recently published, *The Birds of South America* by R. S. Ridgely and G. Tudor, is the first volume in an intended series of four. The initial volume is subtitled *The Oscine Passerines* and encompasses more than 700 species of songbird. Thirty-one colour plates, drawn by Guy Tudor, illustrate the text of Robert Ridgley. This work is seen as a valuable contribution to the recording and appreciation of the birds of South America — especially appropriate at a time when rapid development is devastating millions of acres of tropical habitat in South America. The subsequent volumes of *The Birds of South America* will be: The Suboscine Passerines; The Nonpasserines (Landbirds) and The Nonpasserines (Waterbirds). This illustration is taken from the cover of the first volume, published by Oxford University Press, price £45.

THE subtitle says this book is a "practical calculation manual". Right away, the prospective reader wonders what that could be. Manuals are generally not much fun for off-duty reading. There are many people who might like to read an informative book about bird flight, but few who need to make calculations. The prospective reader wonders if this is a book or a computer manual, and if it's a manual, shouldn't it be someone else's job to read it?

The first chapter doesn't make the question go away. In the very first paragraph, the author launches into a discourse about how millilitres of oxygen and grams of fat are not units of energy. He reveals here, as in other places in the book, that he is on a mission to set some of his colleagues straight about the right way of doing certain things. On p.11, for example, he draws the right and wrong methods of tracing a wing to find its area. On p.8 he says that some collections of data in the literature have to be treated with caution "because ornithologists have proved surprisingly creative in inventing wrong ways to make even the simplest measurements".

Not until the reader is well into the book does it dawn, like a bright light switching on, that this volume — manual or not — represents a beautiful and original achievement. From then on, it doesn't matter whether it is a book, a manual or a computer program (actually, it is all three). What matters is that Pennycuick has invented something here; a way of exploring a complex biological subject through first-hand revelations using a calculation model.

It is a very successful invention. Books are good tools for self-education, but Pennycuick obviously believes there are better ways to learn. The best way is not to be told the answer but to be given the opportunity to discover it for oneself. Pennycuick's invention is not at all like computer-aided instruction, where the program asks the user a question and then scolds and corrects when it thinks the answer is wrong. Using the programs provided here, the user is responsible for formulating a scientific question. An example (p.88) might be: why do birds of intermediate size use a mode of intermittently powered flight (bounding) in which the wings are folded against the body during the non-flapping phase? The user can work out answers to such questions by obtaining measurements, not on animals, but from a calculated simulation of what the animal would do in the presumed situation. Most of the book concerns explanations of how the programs arrive at their answers. Pennycuick provides brief discussions of the physical and biological principles behind the calculations, emphasizing throughout the importance of selecting and configuring the program for the task at hand and staying within the limitations inherent in the program's assumptions. He has made it straightforward to change the assumptions when necessary, as, for example, when the air density is lower because the bird is flying at altitude.

The book is full of fresh insights I have never seen published. I liked the very simple and clear discussion of Reynolds number on p.43, where the author points out that the ratio of inertial force to viscous force acting on the air in a little cube flowing around an obstruction grows larger if you scale the whole drawing up because the ratio of the cube's mass to its surface area increases. On p.50 I was told that a snow goose body in a wind tunnel has 15 per cent less drag when the feathers are coated by hair spray. In a living bird, the feathers can be controlled by pteromotor muscles, presumably giving some of the same effect. On p.62, there is a diagram of the concertina wake, in which the bird flexes and shortens its wings on the upstroke while flapping, so that no transverse vortices are shed, and consequently less energy is required than would be the case for flapping wings of fixed span.

A particularly nice use of the programs included with the book is discussed on p.107, where the author asks why small birds don't take advantage of the energy in the atmosphere and soar as large birds do. He shows how his programs can produce a telling calculation comparing a 5.5-kilogram crane to a 17.4-gram ovenbird, assuming both birds have 1 metre per second thermals to soar in. Whereas the crane requires only 15 per cent as much energy per kilometre to migrate using thermals as to flap along in still air, the little ovenbird uses 6 per cent *more* energy per kilometre riding the thermals. The reason the small bird comes out so badly is that its basal metabolism is a much larger fraction of the total energy requirement. Because the small bird spends almost three times as long using thermals as it would flying direct, the extra time results in an extra cost for basal metabolism that overwhelms the energy savings that would be available from gliding instead of flapping. ☐

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presumed rules, and that accounts for evolution It is not clear how a primaeval bacterium can have the incipient structure of both a mammal and an angiosperm, but I assume that these laws of structure allow for — even require — a rigid, prescribed sequence of change It is also possible, as some of the authors suggest in this symposium volume, that one might have a mixture of natural selection and these laws of structural transformation, the latter dealing with the important changes in form It is argued, for instance by M Ito, that convergence in evolution is not explained by the standard idea of similar selection pressures producing similar structures, but that the reverse is true the primordial structure is the same and that is why different kinds of organisms produce the same shapes There is even the interesting suggestion by K Ikeda that these structural rules might be exerting their influence directly on the DNA of the genome in the form of super control elements

The problem of development receives considerable attention from a number of authors, especially Brian Goodwin He seeks these laws of structure and asks whether perhaps some of the reaction-diffusion models of development inspired by Alan Turing, or the mechanochemical model of Murry, Odell, Oster and others, might lead us into the structural laws The arguments for this and other more directly genetic influences on development are intelligent and sophisticated, but Goodwin has not made me a convert For me, the quest of the grail of developmental biology is not some hidden set of laws, but a simple unravelling of the web of mechanisms that control development I see the experimentalist who does developmental genetics as being on the right track, and there is much in current progress to encourage that view

There is another aspect to structure and evolution development that should be mentioned Many authors have recently pointed out that organisms can only build in one generation, or change over many, if they use the building blocks which are available to them They have called these 'constraints' and made much of the obvious point that neither evolution nor development can make wildly abrupt morphological shifts It is in these very constraints where structuralists see the hidden power that will explain all

To return to the volume, let me say that it is a mixed bag of essays Gerry Webster introduces the topic and many of the papers directly address the idea of structuralism, some clearly also embracing darwinism A few are wonderfully impenetrable, while others, such as the short paper of K Ikeda, are models of clarity

D'Arcy Thompson's *On Growth and Form* is often referred to in the book, and this adds yet another facet to the psychological problem For Thompson a satisfying explanation was a mathematical description I understand and respect this, but it does not satisfy me For the structuralist there is an unknown set of rules that govern form, but that does not satisfy me either, for I feel they may not exist and certainly it is unlikely that we will ever find out what they are I am quite happy with vulgar natural selection and developmental genetics ☐

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New development

Tim Hunt

Genes and Embryos. Edited by D M Glover and B D Hames *IRL/OUP Press 1989 Pp 228 Hbk £27, $59, pbk £18, $38*

UNDERSTANDING how genes control embryonic development is one of the most important and exciting aims of biology Thomas Hunt Morgan and his colleagues at Columbia University understood this when they provided the basis of modern genetics, but they made only modest progress in discovering how genes actually shape the organism In the fruitfly *Drosophila melanogaster*, however, they chose the right organism to solve their problems, as Morgan's followers have since triumphantly demonstrated The many advances in cell and molecular biology of the past 20 years or so were required, however, to confirm and flesh out the *Theory of the Gene* (Yale University Press, 1926)

But let us not forget that creatures that 'don't have genetics' have also shed light on development amphibians led and arguably continue to lead the way in the study of induction (with nematodes coming up fast on the inside) Good progress (considering the formidable technical problems posed by development within a womb) has been made in describing and analysing mouse embryogenesis Surprisingly, many of the key genes that control development in flies are found in vertebrates too, where they probably work in not-too-dissimilar ways In fact, a tremendous amount is now known, and astonishing principles are emerging at an accelerating pace

Now is a good moment to tell the world about these new understandings People like me sense that these are stirring times but have trouble keeping their segments, parasegments, gap- and pair-rule genes in their correct conceptual slots Stripey flies, mammalian homoeobox genes and growth-factor receptor homologues in worms and flies are frequently reviewed in specialist journals, but it would be wonderful if someone could put it all together and tell, if not *the* story, at least a well illustrated outline of what the story i going to be This will take more than minireview in *Cell* or an article in *Trend in Genetics* Perhaps the nearest approac to this ideal is chapter 16, "Cellular Mechanisms of Development", in *Molecula Biology of the Cell* (eds B Alberts *et al* 2nd edn, Garland Press, 1989)

I had hoped that *Genes and Embryo* was the book I was looking for, but sadly i is not Perhaps its opening sentence give the game away "This book reviews th exciting discoveries made over recen years which contribute to our understanding of development at a molecular level It is the *molecular* that presents th problem, I think, for the authors are al excellent and their organisms apt

Almost half the book is allotted t *Drosophila*, Kathryn Anderson, Michae Levine and Katherine Harding giv commendably up-to-date accounts o developments in this fast-moving an tremendously impressive field But thei densely worded chapters are too sparsel illustrated for easy comprehension M favourite chapter is that by Kennet Kemphues on the nematode worm H achieves a nice balance of backgroun information, methodological explanation shows some striking pictures and provide food for thought Comparatively less i understood of frogs and mice, and it i understandable that the chapters by Tor Sargent and Ian Jackson are increasingl devoted to descriptions of particula genes that happen to have been studie and, particularly for the mouse, t purely methodological considerations Les attempt is made to give an integrated o comparative view of the principles o development of these more complicate organisms Perhaps it is still a bit to soon

The dream book of late twentieth-century embryology thus remains to be written It will need expert authors who meet to argue and refine their ideas and their presentation as well as a sympathetic editor and a gifted illustrator The result will be a fantastic story — one that I had never thought to hear in my lifetime ☐

Tim Hunt is in the Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, UK

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Chemical asymmetric synthesis

John M. Brown & Stephen G. Davies

Thalidomide comes in two forms a left-handed compound which is a powerful tranquilizer, and a right-handed version which can disrupt fetal development, causing severe handicap As a necessary consequence of synthetic methods available in the early 1960s the two forms were present in equal proportions in the manufactured drug, with catastrophic consequences The thalidomide story is perhaps the most painful reminder of the importance of stereochemistry—the spatial ordering of groups in a molecule can be as influential as the chemical nature of the groups themselves A principal problem in organic synthesis is, therefore, the development of methods for producing complex molecules with a stereochemically defined structure

OVER millions of years the complex architectures of enzymes (average relative molecular mass 50,000) have developed into efficient and specific templates for only one hand of organic reactant Since the first synthesis of an organic compound by a chemist in 1824, relatively small and simple reagents (average relative molecular mass <100) have been developed to achieve the synthetic transformations necessary for the construction of organic molecules Despite enormous advances in synthetic methods, which have made the construction of virtually any desired compound feasible, the problems of producing single-handed compounds seemed insurmountable and synthetic compounds were always produced as equal mixtures of left and right hands Recently, however, several breakthroughs in synthesis have occurred whereby more sophisticated, single-handed reagents or catalysts (relative molecular mass <800) have been invented which control the handedness of the product compounds with efficiencies rivalling those of enzymes

In structures of biological interest, such as natural products or synthetic enzyme inhibitors, there are one or more (often several) saturated carbon atoms with four different substituents attached and this tetrahedral arrangement can be either of the possible hands Figure 1 shows the hydration of but-1-ene in which the addition creates such a chiral centre and here, because no selectivity has been imposed, both enantiomers are formed A chemical reagent that is itself chiral may select in favour of one of these, producing a homochiral product If the reactant already possesses a chiral centre then the products, diastereomers, are no longer simply related as object and mirror image and they have different chemical and physical properties This is illustrated in Fig 1*a* by the hydration of homochiral 3-hydroxybut-1-ene The art of asymmetric synthesis is in controlling reactions so that one enantiomer or diastereomer is produced predominantly or exclusively[1-3]

Classical methods for the production of homochiral compounds generally involve some form of resolution experiment, such as Pasteur's manual separation of enantiomorphic crystals or the fractional crystallization of diastereomers[4] If the asymmetric centre is to be introduced by synthesis then one of several different techniques must be applied

- The reagent or reactant may contain a chiral auxiliary, a group in the vicinity of the reaction site that controls the stereochemistry and which is easily removed afterwards, or
- A homochiral catalyst may be used, which can be an enzyme or a synthetic product, or
- A readily available homochiral molecule, often from a natural source, may be incorporated as a building block—the 'chiral pool' approach

Examples of these are shown in Fig 2 The first two are examples of true asymmetric synthesis because chirality is created in the course of reaction, and we will concentrate on such approaches here

The advances in chemical methods of asymmetric synthesis over the past five years[5-7] have been such that the enantiomer excess (% desired −% undesired enantiomer) achieved in a good asymmetric synthesis can be >99%, and 95% is fairly routine Such successful asymmetric syntheses require that information is transmitted efficiently from a pre-existing homochiral source to the reaction site This molecular recognition process involves a combination of attractive forces (electrostatic or van der Waals) and steric repulsion

A high proportion of recent asymmetric syntheses use organometallic or coordination chemistry There are several reasons for this—in particular the ability of metals to complex with the common functional groups in organic molecules in a reversible manner, and the ability to activate simple reactants such as hydrogen and carbon monoxide Also, the reactive complexes are well defined spatially, which is vital for stereochemical control Physicochemical techniques that can elucidate three-dimensional structure are therefore important in developing our understanding of asymmetric synthesis, which is in essence a phenomenon of chemistry in the third dimension Solution spectroscopic techniques, especially nuclear magnetic resonance, are used to some extent X-ray crystallography, however, is still the most important tool, even though asymmetric reactions normally take place in the liquid phase so that an extrapolation from the solid structure is required

Homochiral reagents and auxiliaries

Chirality does not occur spontaneously, it must be imposed during synthesis In general this is achieved using a tightly

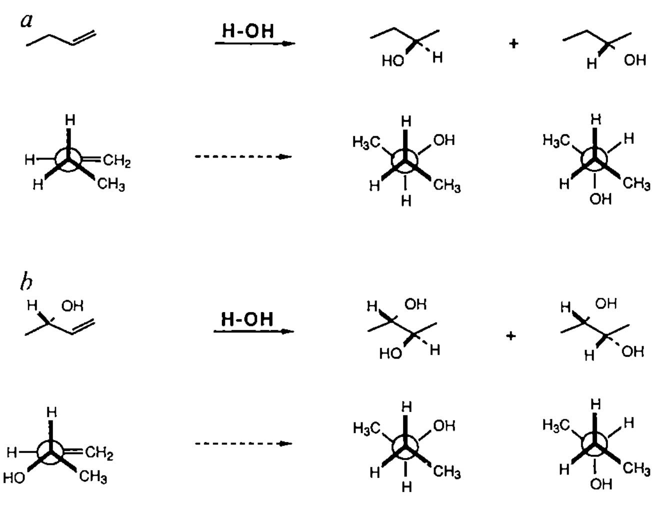

FIG 1 Stereochemistry of hydration of *a*, but-1-ene and *b*, homochiral 3-hydroxybut-1-ene

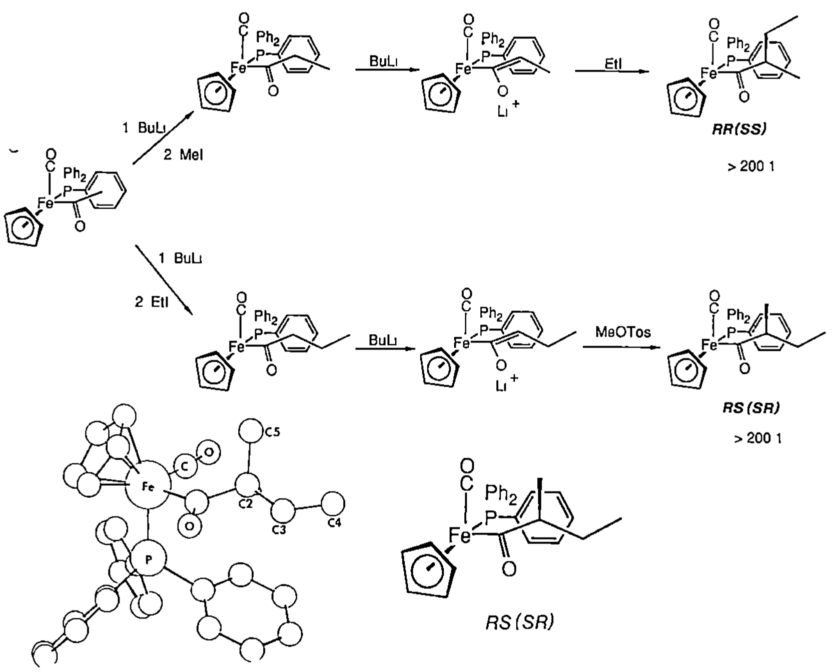

FIG 4 The alkylation pathways for iron enolate complexes, an X-ray crystal structure of the lower product indicating the stereochemistry at C_2 is shown Bu, butyl is C_4H_9, Tos, *p*-toluensulphonyl is $C_7H_7SO_2$

monoxide (very small) This difference in bulk of the ligands in the auxiliary orientates the attached acetyl ligand so as to minimize steric interactions The preferred orientation places the small (relative to methyl) oxygen atom of the acetyl anti to the carbon monoxide ligand, that is, between the large and medium groups (Fig 3) The triphenyl phosphine ligand arranges its phenyls, again to minimize mutual steric interactions, in the form of a rotor Although this rotor is freely rotating, one blade (phenyl group) shields, at all times, one face of the acetyl group (Fig 3) whereas the other face is essentially unencumbered The acetyl group is thus in a fixed orientation in a chiral environment ready for reagents and reactants to approach only from the unhindered face These ingredients are all that are required to impose stereocontrol and achieve asymmetric synthesis

Protons on carbons adjacent to carbonyl groups are acidic[9,12], so removal of a proton from the acetyl, to generate an enolate, is readily achieved with strong base (butyl lithium) Trapping this enolate, which is nucleophilic at the carbon, with the electrophile ethyl iodide forms a new carbon–carbon bond Overall the acetyl group has been converted to the four-carbon butanoyl fragment (Fig 4) The butanoyl group prefers to orientate itself with the terminal two-carbon fragment as far away from the very bulky auxiliary as possible, this exposes only one of the remaining two acidic protons to base Removal of an acidic proton from the butanoyl group generates the corresponding enolate with the ethyl group *cis* to the enolate oxygen Trapping this enolate with the electrophile methyl iodide forms a new carbon–carbon bond and generates the 2-methylbutanoyl complex (Fig 4) The addition of the methyl group can only occur from the unhindered face of the enolate thus giving rise to the stereocontrolled formation of a new chiral centre Figure 4 also shows the X-ray crystal structure of the product 2-methylbutanoyl complex The chiral auxiliary maintains the same structural features as before Looking at this structure, it should be easy to envisage the controlled construction of the chiral centre The final step involved the formation of the C_2–C_5 bond with C_5 approaching C_2 away from the triphenylphosphine and C_3 and C_4 away from the iron Overall two of the hydrogens of the original acetyl group have been replaced sequentially by different organic fragments The method is very versatile, the stereocontrol is very good and the enantiomer produced for a given enantiomer of the chiral auxiliary depends only on the

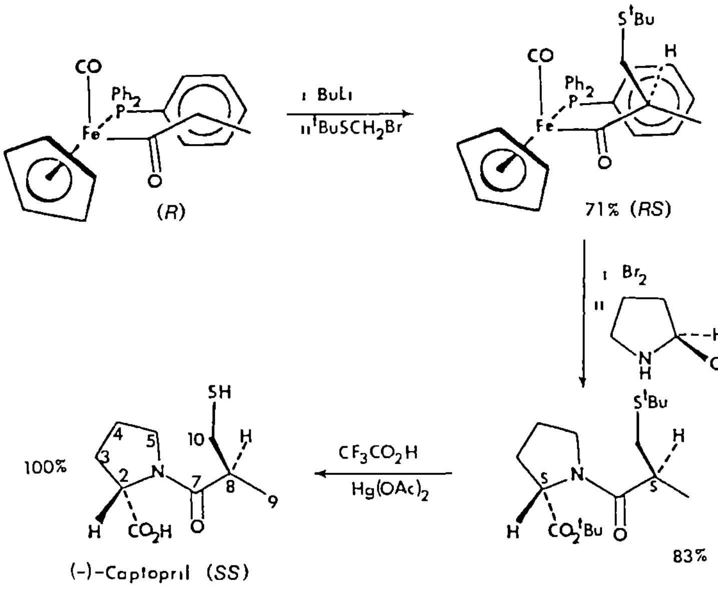

FIG 5 The iron acyl route to *SS*-captopril Ac, acetyl is $COCH_3$

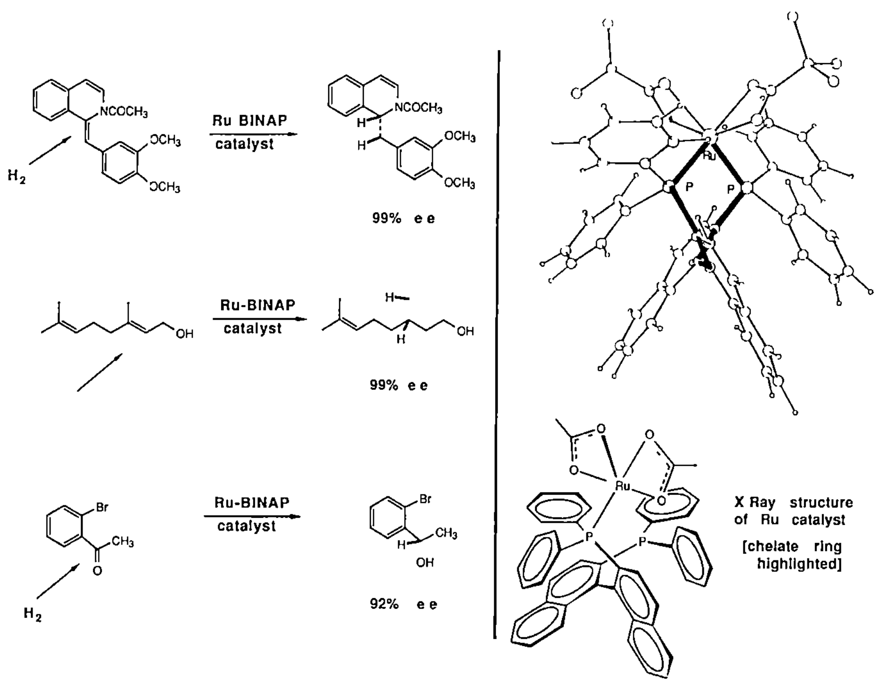

FIG 6 Ruthenium asymmetric hydrogenation with the BINAP complex shown e e, enantiomer excess

sequence of the carbon–carbon bond forming steps

Removal of the chiral auxiliary is readily achieved by one-electron oxidants such as bromine Oxidation in the presence of water, alcohols or amines leads to the formation of carboxylic acids, esters and amides respectively The stereochemical integrity of the new chiral centre is not compromised in the release from the chiral auxiliary[8–10]

The asymmetric synthesis of (−)-captopril is illustrated in Fig 5 Starting from the parent iron acetyl complex the required chiral centre is constructed as before by the sequential introduction of a methyl and a protected thio hydroxymethyl group Removal of the auxiliary in the presence of the *t*-butyl ester of L-proline forms the required amide bond to yield doubly protected captopril Simple deprotection releases (−)-captopril that is identical in all respect to an authentic sample

Homochiral catalysts

Enzymes in synthesis Although we focus on chemical rather than biochemical methods, we consider the latter briefly here Purified enzymes can catalyse the right asymmetric syntheses with near-perfect specificity This applies not only to their natural substrates but to reactants with sufficient structural similarity that the active site recognizes them in the same way Because many important enzymes are readily available—hydrogenases($C{=}O \rightarrow CHOH$), esterases($COOR \rightarrow COOH$) and aldolases($RCHO \rightarrow RCH(OH)CH_2COR'$), for example—biocatalysis will have a significant role in the development of asymmetric synthesis[13–18] At present the limitations are twofold first, the range of substrates is constrained by the natural function of the enzyme, second, the reaction must normally (but not always) be carried out in dilute aqueous solution It is a rapidly developing area, and site-specific mutagenesis may help to diminish these limitations

Asymmetric hydrogenation[19,20]**.** Among reactions catalysed by transition-metal complexes, the addition of hydrogen to multiple bonds is both the most widely used and the best understood Before 1970 G Wilkinson and co-workers had demonstrated that rhodium phosphine complexes were efficient catalysts for

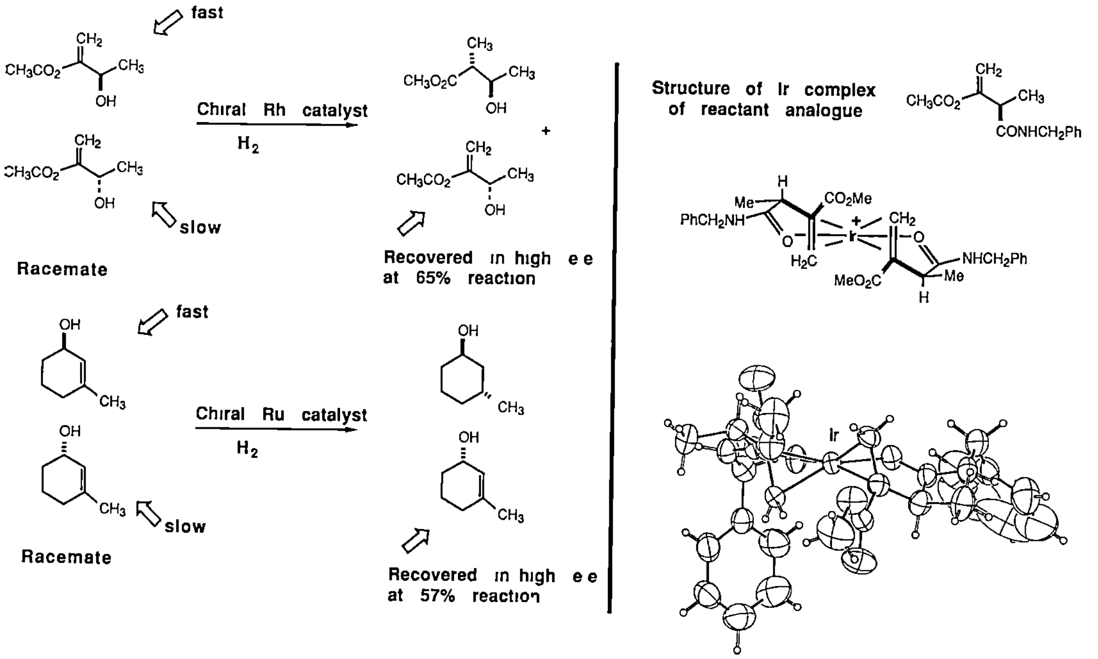

FIG 7 Catalytic kinetic resolution in hydrogenation with rhodium and ruthenium complexes

he reaction in organic solvents[21] The development of homogeneous hydrogenation was a principal factor in the award of he 1973 Nobel prize to Wilkinson Many groups realized he potential of this reaction in asymmetric synthesis By 1977 t had been discovered that the double bond of dehydroamino acids could be hydrogenated to one enantiomer of the amino acid with essentially complete specificity The critical insight, for which major credit must be given to H B Kagan and W S Knowles, was the possibility of using chelating biphosphines as chiral ligands[19,20] The recognition process involves coordination of the dehydroamino acid (introduced as an amide) to hodium through both the olefin and amide group The presence of a secondary binding site is a familiar concept to enzymologists and it proves to be a critical feature for asymmetric synthesis Thus rhodium asymmetric hydrogenation is not a general reaction—substrates that lack secondary binding are not reduced with high enantioselectivity Nevertheless, this reaction remains one of the best chemical routes to optically pure amino acids of D- or L-configuration and was used in an industrial-scale synthesis of L-DOPA (for clinical treatment of Parkinson's disease) for several years Many homochiral ligands are effective provided that they can form chelates, and they probably operate by a common mechanism[20]

The next major breakthrough came in 1986-7 In early work, Wilkinson, James and others had shown that ruthenium complexes were effective for the catalytic hydrogenation of simple olefins[21] The field was not developed to the same extent as rhodium catalysis until the group led by R Noyori prepared ruthenium complexes of the homochiral ligand BINAP (most ligands for asymmetric catalysis have unwieldy chemical names so acronyms are used) These have proved to give extraordinary specificity for a broad range of asymmetric reductions[22 23] The reaction is not confined to olefins, for many unsymmetrical ketones can be hydrogenated to homochiral alcohols Some typical reaction sequences are illustrated in Fig 6, along with a crystal structure of the catalyst precursor, a biphosphine ruthenium bis-acetate The reason for its efficacy is not yet properly understood, but the ligand can be viewed as a chiral scaffold, with the P-aryl rings rather rigidly defined in space, occupying two coordination sites of hexacoordinated ruthenium In the catalytic cycle the acetates are displaced so that four coordination sites are made available Three sites are taken up by two adjacent hydrides and by the auxiliary binding group The remaining site is available for the olefin or ketone such that only one of its two faces can be accommodated, leading to the observed specificity

Catalytic kinetic resolution[24] The examples of asymmetric hydrogenation given above involved substrates that have an auxiliary binding group in the vicinity of the reduction site If this is itself at an asymmetric centre, then further stereochemical control can be exercised With any catalyst, chiral or otherwise, the new asymmetric centre may bear a defined stereochemical relationship to the existing one—the binding group may direct the course of reduction The cationic rhodium complexes that are effective for asymmetric hydrogenation also work well in such directed hydrogenations, with better than 99% of one diastereomer produced[25,26] Further, the substrate tolerance is much greater than in the former reaction, so that hydroxy, carboxy and acylamino are all efficient directing groups

The course of these directed hydrogenations is controlled by steric effects that result because of the binding to rhodium The result can be predicted from molecular models which clearly show that coordination of one face of the olefin is strongly unfavourable on steric grounds The idea is reinforced by an X-ray structure of a typical reactant coordinated to iridium—it has the same relative configuration as the product of directed hydrogenation (Fig 7) (N W Alcock, J M B, A Conn, A P James and R J Taylor, manuscript in preparation)

If the chiral starting material is racemic (equal amounts of both hands) and the catalyst homochiral then kinetic resolution may occur This requires that the two hands of substrate react at sufficiently different rates In this case the fast-reacting enantiomer is increasingly depleted as reaction proceeds, and the starting material tends towards optical purity The relative rate of fast and slow-reacting enantiomers is expressed as a selectivity factor *S*, for an *S* value of 50 an enantiomer excess of 95% is obtained at 55% reaction (45% recovery of optically active starting material) whereas an *S* value of 10 leads to an enantiomer excess of 95% at 65% reaction (35% recovery of starting material) For the reductions catalysed by rhodium complexes, shown in Fig 7, *S* values of between 10 and 25 have been recorded, making this an effective route to optically enriched derivatives of acrylic acid, a useful building block in synthesis

The ruthenium complexes discussed earlier have similar efficiency in kinetic resolution of acylates, but require hydrogen pressures greater than 1 atmosphere They can also be used for the kinetic resolution of some simple cycloalkenols (see Fig 7) where the rhodium catalysts are ineffective

Catalytic amplification of chirality The reaction of organometallic reagents with aldehydes is one of the most common methods for the formation of a new carbon–carbon bond It was discovered recently that organozinc reagents such as Et$_2$Zn will

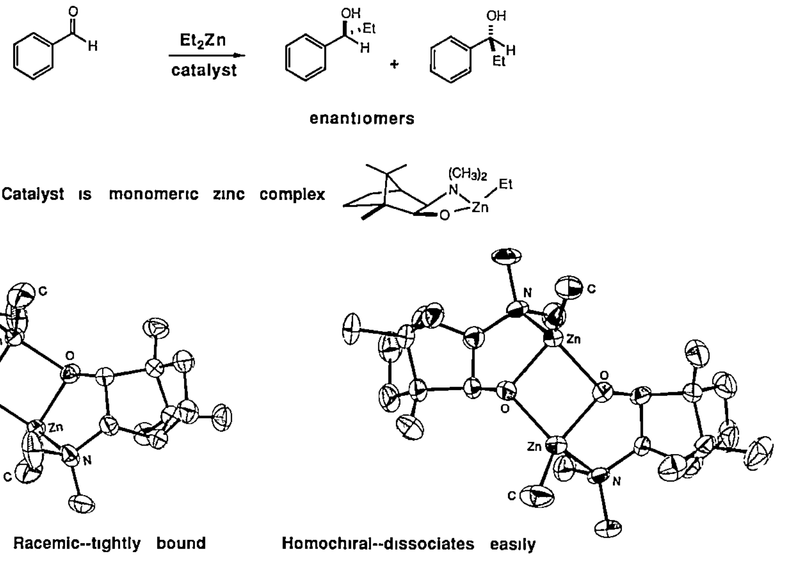
O
H
Et$_2$Zn
catalyst
OH
Et
H
+
OH
H
Et
enantiomers
Catalyst is monomeric zinc complex
(CH$_3$)$_2$
N
Et
O
Zn
N
C
Zn
O
O
Zn
N
C
N
C
Zn
O
O
Zn
C
N
Racemic--tightly bound
Homochiral--dissociates easily
X-ray structures of complexed methylzinc dimers

FIG 8 Aminoalcohol-catalysed addition of diethylzinc to aldehydes

add to aldehydes in the presence of a catalytic amount of a β-aminoalcohol[27-30] Further, if the catalyst is homochiral, the resulting secondary alcohol is formed with a high enantiomer excess This proves to be a valuable procedure, especially for aromatic products The catalysts can be derived from the natural products camphor, proline or ephedrine by conventional chemical synthesis

The catalyst for this reaction forms a complex with the organozinc reagent that has been isolated in crystalline form It proves to be a dimer that is characterized by a central core unit—a four-membered ring of alternating zinc and oxygen atoms This dimer possesses a C_2 symmetry axis Noyori's group also prepared the corresponding racemic dimer which has a similar structure but with a plane of symmetry because it contains one molecule of each enantiomer This has also been characterized by X-ray crystallography (Fig 8) In solution, the binding constant for formation of the racemic dimer is very much greater than that for formation of the optically active dimer Conversely, the racemic complex dissociates to monomer much less readily For the catalytic addition reaction, it is the monomeric complex formed by dissociation of the dimer which is the active catalyst Hence the optically active complex is a much more reactive catalyst than the racemic complex

These observations have been exploited in a novel experiment in which chirality is apparently created[31,32] Consider a catalytic zinc complex in which the initial enantiomer excess of the β-aminoalcohol is 10% Most of this will be present in solution as racemic dimer which is a rather unreactive catalyst This leaves a small amount of homochiral dimer which readily dissociates to a reactive catalyst for the aldehyde addition reaction Consequently, the optical yield is almost as high as that obtained with a homochiral catalyst This apparent conjuring trick has no immediate practical importance but may help in the design of the next generation of asymmetric catalysts It has an obvious relevance to the debate on the primordial origins of chirality, because it demonstrates an amplification process with a purely chemical mechanism

The future

The differing pharmacological effects of the two enantiomers of chiral molecules are now well documented Even if the inactive enantiomer lacks undesirable side effects, administering th potent enantiomer alone halves the dose and alleviates stres on the patient's already compromised metabolism We are a the watershed of asymmetric synthesis—in the near future will be common practice to synthesize all potential new drug as single enantiomers and there is already pressure from regulatory agencies in this direction

Although we have concentrated on drugs in the above articl many other exciting applications are unfolding, now that th synthesis of single enantiomers is becoming more general Th bulk properties of polymers constructed from single enantiomer of monomers are very different from those constructed from racemates A requirement of new materials for molecular electronics is that they be non-centrosymmetric This is, b definition, a property of enantiomerically pure materials Futur generations of, for example, optical data storage devices ma depend on enantiomerically pure organic compounds

The recent advances in chemical asymmetric synthesis tha we have reported here need to be compared to enzyme methods in which there have been recent developments in site-directe mutagenesis The two approaches are complementary and wil contribute in distinct ways As a broad generalization, th methods of biotechnology are most suited to the production o natural molecules, or of closely related homochiral compounds and spectacular selectivity can be achieved By contrast, potentially all homochiral compounds are accessible in enantiomerically pure form by chemical asymmetric synthesis

In many ways the chemical methods described here are stil quite primitive There is still much to be learned about th recognition processes which are at the core of asymmetric synthesis, and the trend will be towards tailoring the structure o reagents and catalysts to take account of increased understanding gained through mechanistic, structural and compute modelling experiments The strength lies in versatility, becaus the only constraint on design of these reagents and catalysts i the ingenuity of the research scientist We expect that the nex decade will see advances in chemical approaches to asymmetri synthesis that will outstrip biological methods in terms o efficiency, versatility and output □

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1 Pratt A J Davies S G Brown J M & Fleet G W J *Chemy Br* **25**, 259-268 (1989)
2 Morrison J D (ed) *Asymmetric Synthesis* Vol 1-5 (Wiley New York 1983-5)
3 Brunner H *Topics Stereochem* **18**, 129-248 (1988)
4 Jacques J Wilen S H & Collet A *Enantiomers Racemates and Resolutions* (Wiley New York 1981)
5 Evans D A Chapman K T & Bisaha J *J Am chem Soc* **110**, 1238-1256 (1988)
6 Gao Y *et al J Am chem Soc* **109**, 5765-5777 (1987)
7 Fleet G W J Ransden N G & Witty D R *Tetrahedron* **45**, 327-336 (1989)
8 Davies S G *et al Phil Trans R Soc* **A326**, 619-631 (1988)
9 Davies S G *Pure appl Chem* **60**, 13-20 (1988)
10 Davies S G *et al Bull Soc chim Fr* 608-630 (1987)
11 Bashiardes G & Davies S G *Tetrahedron Lett.* **28**, 5563-5564 (1987)
12 Brown S L Davies S G Foster D F Seeman J I & Warner P *Tetrahedron Lett* **27**, 623-626 (1986)
13 Porter R & Clark S (eds) *Enzymes in Organic Synthesis Ciba Foundation Symp 111* (Pitman London 1985)
14 Schneider M P (ed) *Enzymes as Catalysts in Organic Synthesis* (Riedel Dordrecht 1986)
15 Jones J B *Tetrahedron* **42**, 3351-3400 (1986)
16 Yamada H & Shimizu S *Angew Chem Int Edn Engl* **27**, 622-640 (1988)
17 Pratt A J *Chemy Br* **25**, 282-287 (1989)
18 Bednarski M D *et al J Am chem Soc* **111**, 627-635 (1989)
19 Kagan H B *Comprehensive Organometallic Chemistry* Vol 8 560-600 (eds Wilkinson G Stone F G A & Abel E W) (Pergamon Oxford 1982)
20 Brown J M & Chaloner P *Catalysis with Metal-Phosphine Complexes* Ch 4 131-170 (ed Pignolet L) (Plenum New York 1984)
21 James B R *Comprehensive Organometallic Chemistry* Vol 8 Ch 51 (eds Wilkinson G Stone F G A & Abel E W) (Pergamon Oxford 1982)
22 Takaya H *et al J Am chem Soc* **109**, 1596-1598 (1987)
23 Kitamura M *et al J Am chem Soc* **110**, 629-631 (1988)
24 Kagan H B & Fiaud J-C *Topics Stereochem* **18**, 249-330 (1988)
25 Brown J M Cutting I & James A P *Bull Soc chim Fr* 212-219 (1988)
26 Brown J M *Angew Chem Int Edn Engl* **26**, 191-204 (1987)
27 Oguni N & Omi T *Tetrahedron Lett* **25**, 2823-2827 (1984)
28 Kitamura M Suga S Kawai K & Noyori R *J Am chem Soc* **108**, 6071-6072 (1986)
29 Soai K Ookawa A Kaba T & Ogawa K *J Am chem Soc* **109**, 7111-7119 (1987)
30 Noyori R Suga S Kawai K Okada S & Kitamura M *Pure appl Chem* **60**, 1597-1612 (1988)
31 Oguni N Matsuda Y & Kaneko T *J Am chem Soc* **110**, 7877-7879 (1988)
32 Kitamura A Okada S Suga S & Noyori R *J Am chem Soc* **111**, 4028-4036 (1989)

A 17,000-year glacio-eustatic sea level record: influence of glacial melting rates on the Younger Dryas event and deep-ocean circulation

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Coral reefs drilled offshore of Barbados provide the first continuous and detailed record of sea level change during the last deglaciation. The sea level was 121 ± 5 metres below present level during the last glacial maximum. The deglacial sea level rise was not monotonic; rather, it was marked by two intervals of rapid rise. Varying rates of melt-water discharge to the North Atlantic surface ocean dramatically affected North Atlantic deep-water production and oceanic oxygen isotope chemistry. A global oxygen isotope record for ocean water has been calculated from the Barbados sea level curve, allowing separation of the ice volume component common to all oxygen isotope records measured in deep-sea cores.

CHANGES in continental ice volume during the last deglaciation generally have been inferred from two indirect sources: (1) geological mapping of retreating ice margins, and (2) the marine oxygen isotope record. Direct estimates of glacial ice volume based on measured glacio-eustatic sea level changes are well documented from 9,000 years BP (before present)[1], but are poorly constrained before this time. Ruddiman[2,3] recently reviewed evidence supporting three different deglaciation models: (1) the 'smooth deglaciation' model with the most rapid change centred at 11,000 years BP, (2) the 'French two-step' deglaciation model with maximum melting rates from 14,000 to 12,000 years BP and from 10,000 to 7,000 years BP separated by a mid-deglacial pause with little or no ice volume loss, and (3) a 'younger Dryas' deglaciation model, which is distinguished from the French two-step model by a mid-deglacial reversal with significant ice growth from 11,000 to 10,000 years BP. Ruddiman concluded that the available evidence from marine and terrestrial records favoured the smooth deglaciation model. He further suggested that the stepwise decreases of $\delta^{18}O$ $(=([(^{18}O/^{16}O)_{\mathrm{sample}}/(^{18}O/^{16}O)_{\mathrm{standard}}]-1)\times 10^3)$ apparent in many curves may not indicate sudden decreases in ice volume, but instead may be an artefact of a deep-ocean warming.

I sought to test the validity of these models, each with their own important climatic and oceanographic implications, by extending the existing coral-reef sea level curve[1] to the last glacial maximum. The fossil coral reefs surrounding Barbados are a very good system for examining the detail and timing of the last deglaciation. Here I show that the sea level curve derived from the Barbados reefs supports the French two-step model[4]. I then review the evidence for the oceanic and climatic response to this two-step deglaciation, with particular emphasis on understanding the cause of the Younger Dryas event.

Barbados corals

The Caribbean reef-crest coral *Acropora palmata* (Lamarck) is[1,5] the best available sea level indicator for Pleistocene studies. This species is generally restricted to the upper five metres of water and dominates the reef-crest community in many locations. *A. palmata* is one of the fastest growing corals and is often found in an interlocking reef framework on the windward side of Caribbean islands. Its massive size minimizes post-depositional transport and compaction, and its abundance and generally pristine aragonite skeleton make sampling for ^{14}C or $^{230}Th/^{234}U$ age dating relatively easy.

To reconstruct glacio-eustatic sea levels, I chose to core *A. palmata* on the south coast of Barbados for several reasons: (1) the offshore bathymetry is gently sloping compared with the precipitous drop surrounding many Caribbean volcanic islands or the Bahama Bank; (2) Barbados is rimmed by three parallel offshore ridges[6] for which I had extremely detailed bathymetric maps. Macintyre has suggested[6] that these ridges are associated with past sea level changes, comprising potentially thick sequences of *A. palmata*; (3) onshore exposure of the *A. palmata* facies deposited during the penultimate deglaciation[5] (isotope stage 6-5) indicates that the south coast of Barbados is being uplifted at ~34 cm kyr^{-1}. Field mapping of these onshore *A. palmata* deposits indicated that throughout the history of reef growth on Barbados, the most extensive areal and vertical distribution of *A. palmata* occurred on the south and south-east coast; (4) Barbados is located in the western tropical Atlantic where relative sea level is least affected by changes in the equipotential surface that are due to the changing gravitational attraction of the diminishing Northern Hemisphere ice sheet[7]. Possible drawbacks of selecting Barbados include: (1) the assumption that the late Quaternary mean uplift of Barbados is without significant episodic uplift or subsidence; (2) the dip-stick model of using oceanic islands to measure net ocean sea level changes may be too simple or may not apply to Barbados because it is situated on top of an accretionary prism between two oceanic plates. The only adequate way to test these geophysical assumptions is to perform the same drilling and dating experiment at other locations.

Barbados cores and their radiocarbon ages

Between 18 November and 6 December 1988, 16 cores were drilled from the RV *Ranger* offshore of the south coast of Barbados using a wire-line coring rig with a four-point mooring system. The purpose of this cruise was to core a continuous vertical sequence of *A. palmata* deposited during sea level rise associated with the last deglaciation. The stratigraphy of the ten longest cores and the corresponding ^{14}C ages are illustrated in Fig. 1. These results show that a nearly continuous sequence of *A. palmata* was recovered between 7,800 yr BP and 17,100 yr BP.

To help guide site selection three radiocarbon ages were determined while drilling was in progress. The remainder were

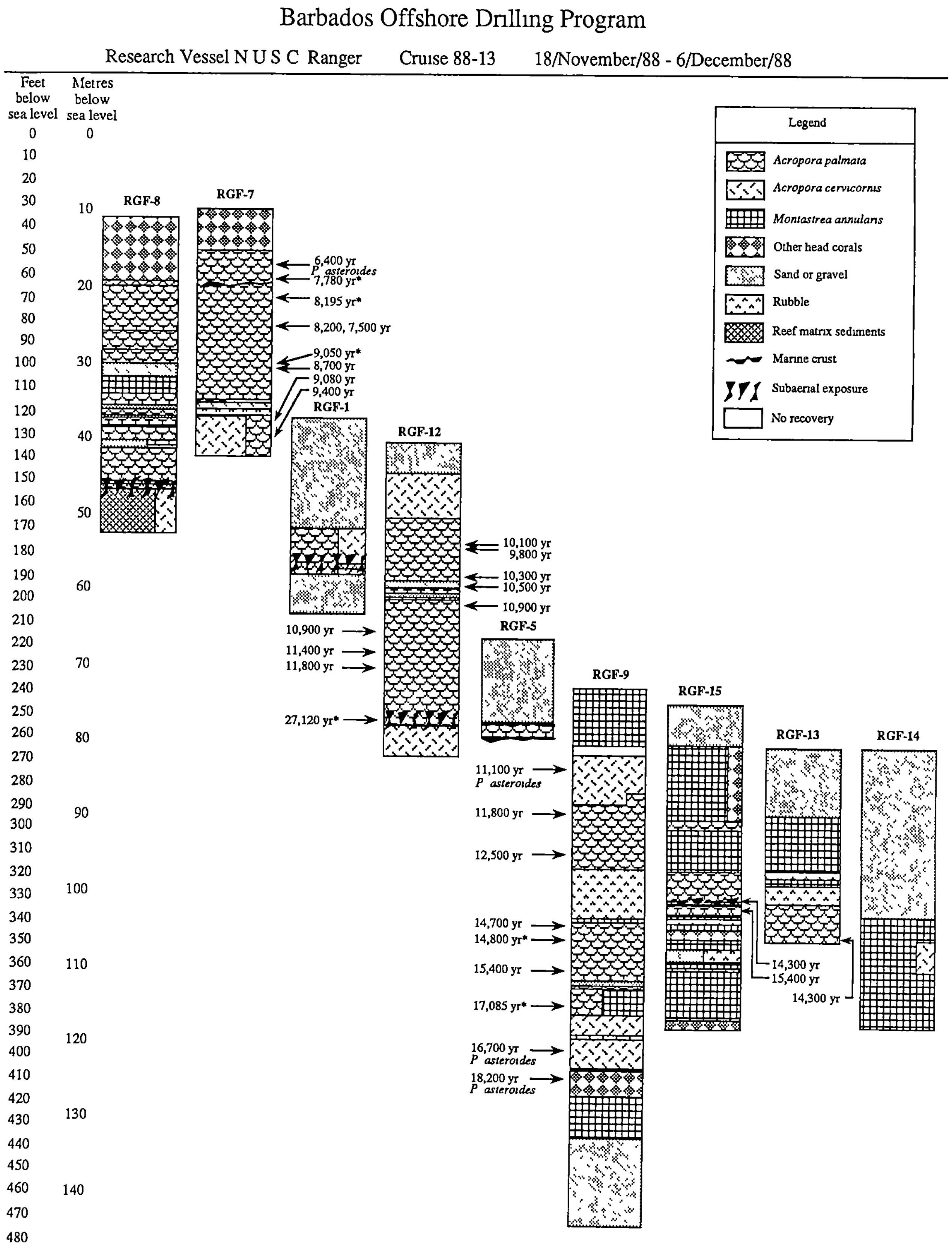

FIG 1 Stratigraphic description of Barbados cores plotted against the modern sea level Radiocarbon ages (yr) of individual corals are indicated by arrows These ages are corrected for local seawater $\Delta^{14}C$ (the difference between the ^{14}C content of air and surface water) by subtracting 400 yr from the measured radiocarbon ages All dated corals are *A palmata* with four exceptions as noted where *P asteroides* were dated Sample at 25 m was dated twice and the 700-yr difference is assumed to arise from a ^{14}C laboratory error The younger age is assumed to be incorrect because it is stratigraphically inconsistent with neighbouring samples This value is therefore not included in Fig 2 Ages marked by an asterisk were measured at Geochron Laboratory, all others at Lamont-Doherty Samples below the subaerial exposure horizons in cores RGF-8 and RGF-1 have been dated at >44,000 radiocarbon years

determined after the cruise using conventional liquid scintillation counting or gas proportional counting The average analytical uncertainty (1σ) of these measurements is ±130 yr for ages less than 17,000 yr All radiocarbon ages are corrected for the apparent age of sea water at Barbados by subtracting 400 yr (ref 8) Radiocarbon ages are not corrected for secular changes in the atmospheric ^{14}C which may be large during the deglaciation[9] As part of a companion study, the ^{230}Th/^{234}U ages of these samples are being determined by mass spectrometry[10] to establish time-dependent changes in the surface ocean ^{14}C levels

The deepest radiocarbon-dated sample of *A palmata* occurs at 113 8 m below the present sea level (119 6 m if corrected for assumed mean uplift rate) and is dated to be 17,100 yr old This age marks the end of the glacial period according to accelerator mass spectrometry (AMS) ^{14}C-dated δ^{18}O records from deep-sea cores[11,12] A sample of *Porites asteroides* from 124 m below the present sea level is 18,200 yr old This date provides a maximum sea level estimate of 131 m (uplift corrected) for the last glacial maximum Unlike *A palmata*, *P asteroides* is not restricted to shallow depths so this glacial maximum sea level estimate must be taken as an extreme Extrapolation of the sea level curve to 18,000 yr BP indicates a sea level of 121 ± 5 m below the present level during the last glacial maximum, the best estimate so far

Between 17,100 and 12,500 yr BP, the sea level increased by 20 m (Fig 2) This first phase of deglaciation was terminated by an exceedingly rapid sea level rise of 24 m in less than 1,000 radiocarbon years, termed melt-water pulse IA (mwp-IA) Melt-water pulse IA (12,000 yr BP) corresponds to the latter part of Termination Ia (14,500–11,500 yr BP) as defined in deep-sea δ^{18}O records (refs 4, 11, later modified in ref 12) Melt-water pulse IA and Termination Ia overlap to some extent, but cannot be synonymous The rate of sea level rise was at a minimum at 11,000 yr BP, marking the beginning of the Younger Dryas chronozone, and remained low until 10,500 yr BP The last half of the Younger Dryas chronozone (10,500–10,000 yr BP) is marked by increasing rates of sea level change, culminating in melt-water pulse IB (mwp-IB) centred at 9,500 yr BP During mwp-IB, which closely corresponds to Termination Ib in deep-sea terminology, the sea level rose ~28 m, similar to the sea level rise associated with mwp-IA

Comparison of seasonal insolation changes

According to the Milankovitch theory[13] the Pleistocene ice ages were caused primarily by changes in the seasonal distribution of incoming radiation associated with orbit variations The Northern Hemisphere ice sheets accumulate in the 40°–70° N latitude range whereas the Antarctic ice sheet is located poleward of 70° S Insolation changes associated with the 41,000-yr tilt cycle increase poleward in both hemispheres, whereas the 23,000-yr precessional cycle dominates insolation changes at middle and low latitudes and is out of phase between hemispheres Since the work of Milankovitch, the time-dependent patterns of radiation have been calculated more precisely[14] The Barbados sea level provides the first direct and high-resolution comparison between the astronomical changes and continental ice response In Fig 3, the changes in insolation over the Laurentide/Fennoscandinavian ice sheet and the Antarctic ice sheet are compared with the melt-water discharge rates calculated from the Barbados sea level curve The discharge rates are plotted in radiocarbon years and must be converted to calender years for comparison with the insolation curves[15] Initial ^{230}Th/^{234}U dates by mass spectrometry indicate that the two melt-water pulses are not artefacts arising from distortions of the radiocarbon timescale (E Bard, B Hamelin and R G F, manuscript in preparation) The ^{230}Th/^{234}U age of mwp-IA is somewhat older, however, than the calibration curve[15] would indicate Although the radiocarbon-age calibration is not well established beyond 9,000 yr BP (ref 15), the absolute-age estimates for the two melt-water pulses are 13,070 and 10,445 calendar years BP based on the ^{14}C calibration of ref 15 This comparison shows that the most intense interval of ice-sheet disintegration (13,070 calendar years BP) predates the Northern Hemisphere insolation maximum (centred at 11,000 years BP) by ~2,000 yr (Fig 3) Melt-water pulse IB is 500 years younger than the insolation maximum At present there is no astronomical explanation for separate melt-water pulses The distribution of Northern Hemisphere ice and the retreat of its margins was not uniform[16], and the relative contribution and timing of Antarctic ice decay is not known These as well as other factors, such as changing ocean and atmosphere circulation patterns, may explain the variable discharge rates

The global oxygen isotope curve

The ^{14}C-dated Barbados sea level curve can be scaled to δ^{18}O using the calibration of 0 011‰ per metre change in sea level[5] (Fig 2) This calibration is a maximum value and corresponds to a mean glacial-ice δ^{18}O value of −42‰ (standard mean ocean water) Based on ice-core δ^{18}O data from Greenland and Antarctica and modern δ^{18}O measurements in precipitation, this value provides a reasonable upper limit for mean ice composition for the entire deglaciation The global δ^{18}O curve predicted from the Barbados sea level curve represents the ice volume component common to all δ^{18}O records from deep-sea cores After taking into account the mixing time of the ocean, the global δ^{18}O curve can be subtracted from any high-resolution AMS ^{14}C-dated δ^{18}O curve to compare regional differences in the δ^{18}O of water and temperature

Ocean δ^{18}O record of melt-water discharge rates

During mwp-IA and mwp-IB, glacial melt water discharged (primarily into the North Atlantic surface ocean) at maximum rates of 14,000 km^3 yr^{-1} and 9,500 km^3 yr^{-1} respectively (Fig 3)

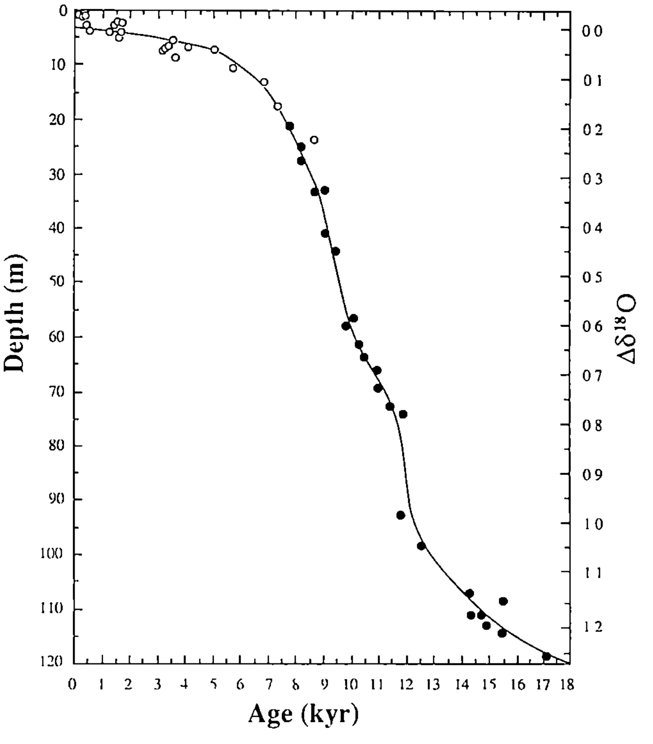

FIG 2 Barbados sea level curve based on radiocarbon-dated *A palmata* (filled circles) compared with *A palmata* age-depth data[1] (open circles) for four other Caribbean island locations All radiocarbon ages in this figure are corrected for local seawater Δ^{14}C by subtracting 400 yr from the measured radiocarbon ages[8] but they are not corrected for secular changes in atmospheric ^{14}C levels[15] The Barbados data are corrected for the estimated mean uplift of 34 cm kyr^{-1} The right-hand axis of the Barbados sea level curve (solid line) is scaled to the estimated δ^{18}O change of mean ocean water using the calibration in ref 5

These glacial-melt-water rates are comparable to the modern fresh-water discharge rate (11,400 km^3 yr^{-1}) into the entire North Atlantic (0–90° N latitude)[17] During the first half of the Younger Dryas chronozone between 11,000 and 10,500 yr BP, melt-water discharge averaged 2,700 km^3 yr^{-1}, a factor of five less than discharge rates during mwp-IA and at least a factor of three less than rates during mwp-IB By comparison, the modern discharge rates for the Mississippi and St Lawrence Rivers are 560 km^3 yr^{-1} and 330 km^3 yr^{-1} respectively[17]

The modern river discharge and marine precipitation is redistributed within the North Atlantic by ocean surface currents and results in strong oxygen isotope gradients of sea water in the north and north-west Atlantic bordering the subtropical gyre Because glacial ice has very low δ^{18}O values relative to sea water, it is reasonable to assume that North Atlantic surface-water δ^{18}O distribution was dramatically affected by the sharp increase in seasonal melt-water discharge during mwp-IA and mwp-IB

The oxygen isotopic composition of planktonic foraminifera is a function of both the ambient seawater oxygen isotopic composition and temperature Foraminiferal species have different seasonal, geographic and vertical distributions whereas melt-water discharge is very seasonal The most extensively dated (AMS ^{14}C) foraminiferal oxygen-isotope record in the North Atlantic is from piston core SU81-18 taken at a depth of 3,135 m near the coast of Portugal (37°46′ N, 10°11′ W)[12] The oxygen isotope record of *G bulloides* shows four distinct steps followed by δ^{18}O minima dated at 13,600, 12,300, 8,800 and 6,800 yr BP (Fig 4) The two largest steps coincide with mwp-IA and mwp-IB Although it is possible that these δ^{18}O shifts with minima at 12,300 and 8,800 yr BP are somehow related to local temperature changes, their coincidence with the salinity (δ^{18}O) minima predicted by the Barbados sea level curve is strong evidence for regional salinity (δ^{18}O) changes I note that lower salinity and warmer temperatures both result in reduced δ^{18}O values of foraminiferal tests Assuming an average glacial melt-water δ^{18}O value of −42‰, then the δ^{18}O of North Atlantic surface water will change at a corresponding rate of −1 2‰ for each 1‰ decrease in salinity Therefore, the δ^{18}O minima in core SU81-18 (Fig 4) can be produced by decreases in salinity of <1‰ The sea level increased only 10 m between 14,300 and 12,500 yr BP, an interval that spans the oldest δ^{18}O minima (13,600 yr) in core SU81-18

Origin of the Younger Dryas event

In the preceding scenario, the Younger Dryas chronozone in the marine realm is characterized by minimal glacial-melt-water discharge, particularly between 11,000 and 10,500 yr BP, and is distinguished as an event primarily by its temporal position between two episodes of lower surface salinity This interpretation of the origin of the Younger Dryas event is fundamentally different from the explanations involving the polar-front movements linked to ice-sheet elevation[2,3,12,18–20] It is the exact opposite of explanations for the Younger Dryas that call upon maximum influxes of glacial ice and water between 11,000 and 10,000 yr BP[18–22]

Apparently there are very different regional responses to the melt-water pulses For example, Mangerud *et al*[23] have shown that in western Norway there were two major glacial re-advances into the North Sea at 12,200 and 10,000 yr BP In the north-east Atlantic, unusually high abundances of the polar species *N pachyderma sin* are found in association with the Vedde ash layer dated at 10,600 years BP (ref 23) This peak abundance of polar species has been interpreted as a Younger Dryas cold event in the north-east Atlantic[18–20] although it apparently coincides with a low melt-water discharge rate Ruddiman *et al*[8] concluded that the Vedde ash, such as found at core site V23-81 on the Feni Drift, was delivered to the north-east Atlantic

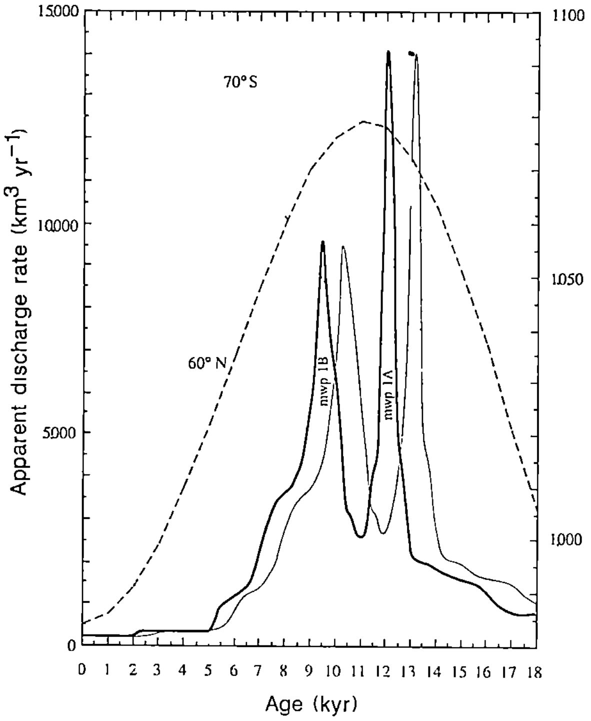

FIG 3 The rate of glacial melt-water discharge calculated from the Barbados sea level curve compared to summer insolation The discharge curve (thick line) is plotted according to radiocarbon years uncorrected for secular changes in atmospheric ^{14}C Converting radiocarbon years BP to calendar years BP using the calibration in ref 15 shifts the ages of mwp-IB and mwp-IA as shown by the thin line Our preliminary ^{230}Th/^{234}U dates indicate that this correction is too small for mwp-IA (E Bard, B Hamelin and R G F, manuscript in preparation) By comparison, the insolation at 60° N (dashed line) and 70° S latitude (dotted line) are plotted against absolute time[14]

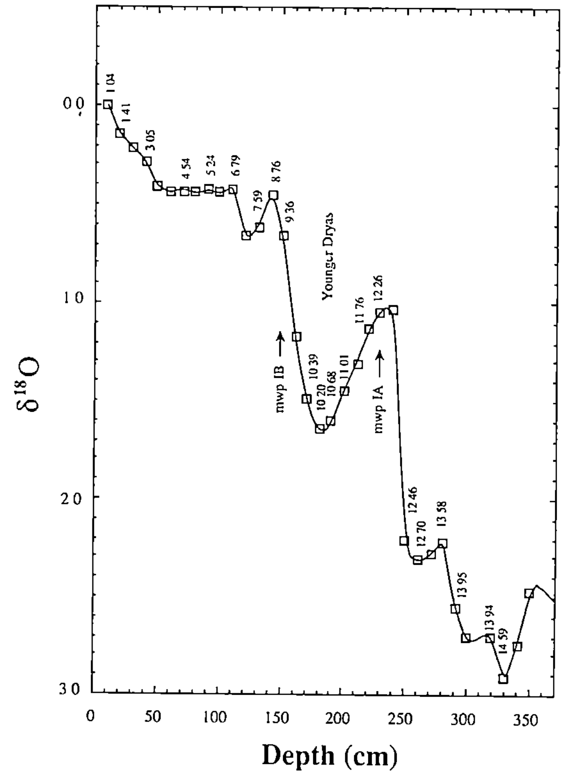

FIG 4 The AMS ^{14}C-dated oxygen isotope record of *G bulloides* from core SU81-18 located offshore of Portugal[12 34] The AMS-^{14}C ages have been corrected for local seawater Δ^{14}C by subtracting 400 yr from the measured radiocarbon ages[8] The estimated depth of mwp-IA and mwp-IB are marked by arrows

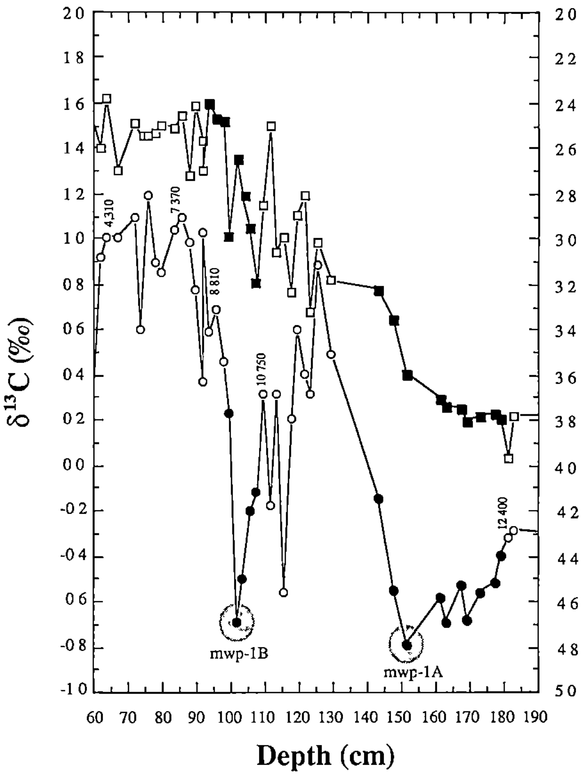

FIG 5 Oxygen and carbon isotope data from core EN120GGC1[29] for the last deglacial interval Age assignments are based on four AMS-^{14}C dates from a companion core that has been stratigraphically correlated to EN120GGC1[30] Using this age model, δ^{13}C minima are approximately coincident with mwp-IA and mwp-IB Closed symbols mark intervals around mwp-IA and mwp-IB and illustrate that δ^{13}C minima occur during the unidirectional shifts in δ^{18}O

by sea ice originating in the Norwegian Sea Recent sea-ice studies[24] show, however, that it is plausible that the *N pachyderma sin* was also imported to the north-east Atlantic by entrapment in sea ice that had formed in the Norwegian Sea

Lower surface-water salinities during mwp-IA and IB are not inconsistent with the faunal distributions in the north-east Atlantic described in, for example, ref 18 Polar station PAPA, at 50° N latitude in the northeastern Pacific, has a low-salinity mixed layer and may be a good analogue for the North Atlantic at 12,000 and 9,500 yr BP Today at station PAPA, an 8 °C seasonal warming of the thin mixed layer results in a seasonal succession of planktonic foraminiferal species from polar to subpolar assemblages[25] Lower salinity might also help explain the evidence for warmer climates on the southern European continent during the Bølling–Allerød and Preboreal periods (see ref 12 for a review) Lower surface salinities generally result in a thinner surface mixed layer with less thermal inertia Energy-balance climate models show[26] that a thinner surface mixed layer in the North Atlantic (>40° N) leads to warmer surface temperatures over land in summer and fall Much larger regional responses over Western Europe are predicted in three-dimensional global-climate-model simulations[26] Conversely, the lack of melt-water stratification of North Atlantic surface waters during the Younger Dryas would allow a return to cooler deglacial conditions recorded by planktonic foraminifera in the North Atlantic ~10,600 yr BP (refs 19, 20, 27) and by western European continental vegetation

Broecker *et al*[27,28] dated the minimum δ^{18}O value (maximum melt-water discharge) in the Gulf of Mexico cores EN32-PC6 and EN32-PC4 at 11,840 and 12,000 yr BP, which are equivalent in age to the AMS ^{14}C-dated δ^{18}O minimum in North Atlantic core SU81-18 (12,260 yr BP)[12] These two events correspond precisely with mwp-IA, dated at 12,000 yr BP (Fig 3) Broecker *et al*[27,28] noted that the maximum glacial-melt-water discharge from the Mississippi River was followed by minimal discharge between 10,500 (EN32-PC6) and 11,100 yr BP (EN32-PC4). They reasoned that this reduction in Mississippi discharge reflected a shift in the drainage from the Mississippi to the St Lawrence River and was not related to changes in glacial melting rates They further speculated that the melt-water diversion caused a salinity decrease in the North Atlantic strong enough to result in a decrease in North Atlantic deep-water formation during the Younger Dryas A reduction in the heat released from the ocean as the northward advection of subtropical surface waters ceased was associated with the reduced formation of North Atlantic deep water The discharge curve derived from the Barbados sea level curve predicts a minimum in Mississippi discharge 11,000 yr BP and therefore it is unnecessary to call upon the unusual combination of events required by the model in refs 27, 28 Furthermore, the discharge curve may explain why no melt-water spike was ever identified in the North Atlantic oxygen isotope records corresponding to an assumed diversion of melt water during the Younger Dryas event

Deep-water response to melt-water pulse

Broecker *et al*[27 28] cite Cd/Ca and δ^{13}C data reported from Bermuda Rise core EN120GGC1[29] as evidence that melt water shut down the production of North Atlantic deep water during the Younger Dryas Boyle and Keigwin's[29] age model for the deglacial section of core EN120GGC1 is based on linear interpolation between age assignments of 14,000 yr BP for Termination Ia and 8,500 yr BP for Termination Ib as well as correlation of the %CaCO$_3$ record with that of a neighbouring core that has two AMS ^{14}C dates on the deglaciation These ages for Terminations Ia and Ib were taken from the AMS ^{14}C dates on North Atlantic core CH73-139C[11] AMS ^{14}C studies on other cores have shown that core CH73-139C suffers from considerable bioturbation[12] The revised ages for Termination Ia are 14,500–12,260 yr BP and for Termination Ib, 10,390–9,360 yr BP (ref 12)

Applying a more accurate chronology to core EN120GGC1[30] shows that the two intervals of highest melt-water discharge are also characterized by extremely low δ^{13}C values, presumably because of reduced influence of North Atlantic deep water at this site (Fig 5) The section corresponding to Younger Dryas interval begins with high δ^{13}C values which then decrease to a minimum value during Termination 1b If this revised timescale for core EN120GGC1 is accurate, then the Younger Dryas and Preboreal chronozones are so condensed that bioturbation must be considered as a significant influence The fact that a relatively subtle adjustment of age assignments can alter the interpretation of deep ocean response to deglaciation emphasizes the necessity for AMS ^{14}C dating of splits of the same samples used for stable isotope and Cd/Ca measurements in core EN120GGC1 Until this is done, it is equally plausible to suggest that the production of North Atlantic deep water was reduced or shut down within the Bølling/Older Dryas (mwp-IA) and Preboreal (mwp-IB) chronozones and not during the Younger Dryas

Continental record of melt-water discharge rates

The rapid disintegration of the Northern Hemisphere ice sheets during mwp-IA and mwp-IB must have introduced large volumes of low-δ^{18}O melt water into the North Atlantic during the summer season This δ^{18}O-spiked melt water has two possible fates entrainment in North Atlantic deep water or evaporation[17] Because deep-water formation may have been halted briefly during the peak discharge, evaporation of δ^{18}O-spiked surface water must have occurred to some extent

The Greenland ice cores Dye 3[31-33] and Camp Century are both marked by two distinct δ^{18}O minima during deglaciation (Fig 6) Many researchers assumed that these δ^{18}O minima indicated cooler temperatures[29-32] and that they were associated with a southward migration of the North Atlantic polar front[19,20] However, recent ^{14}C dating of air bubbles in the Dye 3 ice core[33] enables a direct comparison to the ^{14}C dated Barbados sea level curve (Fig 6) Six AMS ^{14}C measurements were made on samples taken at depths between 1,660 and 1,780 m (ref 33) Using the age-depth relationship (squared correlation coefficient = 0 93) determined from these data, the estimated age for the δ^{18}O minimum at 1,792 m (Fig 6) is 9,320 ^{14}C-years BP The age of this δ^{18}O minimum in Dye 3 matches the age for mwp-IB (9,500 ^{14}C-years BP) It is not clear whether the ^{14}C age is concordant with the estimated calendar age based upon annual layer counts[31], because the radiocarbon timescale is not well calibrated in this time range I believe that these presumed temperature minima in the Greenland δ^{18}O records may in fact be the result of source-water variability as well as temperature If so, they may be the terrestrial equivalents of δ^{18}O minima in deep-sea cores such as SU81-18 (Fig 4)

The immediate implications of the Barbados sea level record compel us to re-evaluate some of the traditional depictions of the Younger Dryas event Whether or not the reinterpretations presented here are correct depends on the reliability of correlations between radiocarbon-dated marine and terrestrial signals My results emphasize the primary role of melt-water discharge rates on the production of North Atlantic deep water and rapid climate change in western Europe ☐

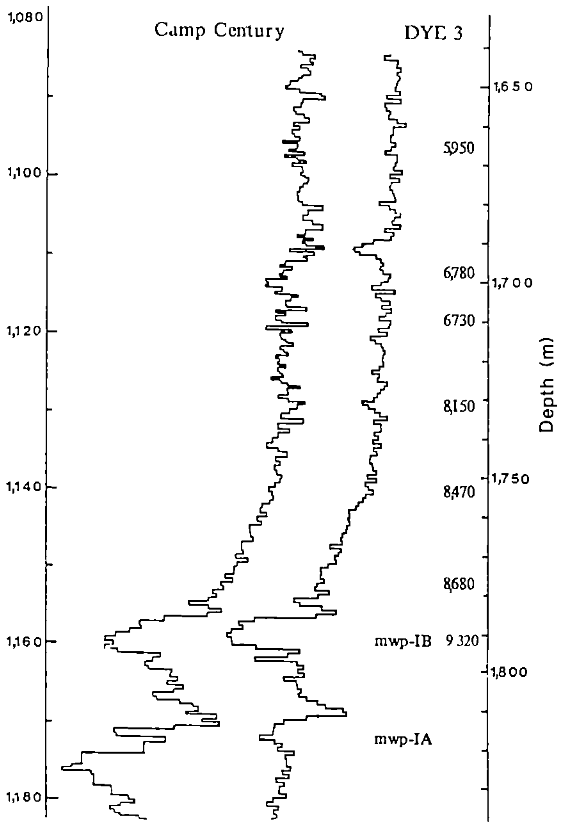

FIG 6 Plot of terrestrial δ^{18}O records from Greenland ice cores Dye 3 and Camp Century[31 32] with six radiocarbon ages[33] indicated for Dye 3 Age for mwp-IB is estimated from the ^{14}C-depth relationship[33]

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1 Lighty R G Macintyre I G & Stuckenrath R *Coral Reefs* **1**, 125-130 (1982)
2 Ruddiman W F in *North American and Adjacent Oceans During the Last Deglaciation* (eds Ruddiman W F & Wright H E Jr) **K-3** 463-478 (Geol Soc Am Boulder 1987)
3 Ruddiman W F in *North American and Adjacent Oceans During the Last Deglaciation* (eds Ruddiman W F & Wright H E Jr) **K 3** 137-154 (Geol Soc Am Boulder 1987)
4 Duplessy J C Delibrias G Turon J L Pujol C & Duprat J *Palaeogeogr Palaeoclimatol Palaeoecol* **35**, 121-144 (1981)
5 Fairbanks R G & Matthews R K *Quat Res* **10**, 181-196 (1978)
6 Macintyre I G *Am Ass Petrol Geol Bull* **56**, 720-738 (1972)
7 Clark J A Farrell W E & Peltier W R *Quat Res* **9**, 265-287 (1978)
8 Bard E *Palaeoceanography* **3**, 635-645 (1988)
9 Oeschger H *et al Radiocarbon* **22**, 299-310 (1980)
10 Edwards R L Chen J H Ku T L & Wasserburg G L *Science* **236**, 1547-1553 (1987)
11 Duplessy J C *et al Nature* **320**, 350-352 (1986)
12 Bard E *et al Nature* **328**, 791-794 (1987)
13 Milankovitch M M *Canon of Insolation and The Ice-Age problem* (Koniglich Serbische Akademie Beograd 1941) English tranlation published by the U S Department of Commerce and the NSF Washington DC
14 Berger A L *Quat Res* **35**, 215-240 (1978)
15 Stuiver M *et al Radiocarbon* **28**, 969-979 (1986)
16 Andrews J T in *North American and Adjacent Oceans During the Last Deglaciation* (eds Ruddiman W F & Wright H E Jr) **K 3** 13-38 (Geol Soc Am Boulder 1987)
17 Baumgartner A & Reichel E *The World Water Balance* (Elsevier New York 1975)
18 Ruddiman W F Sancetta C D & McIntyre A *Phil Trans R Soc* **B280**, 119-142 (1977)
19 Ruddiman W F & McIntyre A *Science* **212**, 617-627 (1981)
20 Ruddiman W F & McIntyre A *Palaeogeogr Palaeoclimatol Palaeoecol* **35**, 145-214 (1981)
21 Mercer J H *Arctic Alpine Res* **1**, 227-234 (1969)
22 Johnson R G & McClure B T *Quat Res* **6**, 325-353 (1976)
23 Mangerud J Lie S E Furnes H Kristiansen I L & Lomo L *Quat Res* **21**, 85-104 (1984)
24 Spindler M & Dieckmann G S *Polar Biol* **5**, 185-191 (1986)
25 Reynolds L & Thunell R C *J Foraminiferal Res* **15**, 282-301 (1985)
26 Schneider S H Peteet D M & North G R in *Abrupt Climate Change* (eds Berger W H & Labeyrie L D) 399-418 (Reidel Dordrecht 1987)
27 Broecker W S *et al Palaeoceanography* **3**, 1-19 (1988)
28 Broecker W S *et al Nature* **341**, 318-321 (1989)
29 Boyle E A & Keigwin L *Nature* **330**, 35-40 (1987)
30 Keigwin L D Jones G A *Deep Sea Res* **36**, 845-867 (1989)
31 Hammer C U Clausen H B & Tauber H *Radiocarbon* **28**, 284-291 (1986)
32 Dansgaard W White J W & Johnson S J *Nature* **339**, 532-534 (1989)
33 Andree M *et al Radiocarbon* **28**, 417-423 (1986)
34 Bard E *Quat Res* **31**, 381-391 (1989)

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Cloning by functional expression of a member of the glutamate receptor family

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We have isolated a complementary DNA clone by screening a rat brain cDNA library for expression of kainate-gated ion channels in Xenopus oocytes The cDNA encodes a single protein of relative molecular mass (M_r) 99,800 which on expression in oocytes forms a functional ion channel possessing the electrophysiological and pharmacological properties of the kainate subtype of the glutamate receptor family in the mammalian central nervous system

THE synapse plays a key role in the nervous system, and it is likely that biochemical changes at the synapse underlie some aspects of higher brain function Most plausible theories of learning, for example, depend upon changes in the efficiency of chemical synapses[1,2] Glutamate receptors are believed to play a central part both in memory aquisition and the establishment of "topographical maps"[3,4] Hence, this receptor family has become the focus of much experimental work Glutamate receptors are found throughout the mammalian brain and represent the predominant excitatory neurotransmitter system[3] The best-studied glutamate receptors are ligand-gated ion channels that are permeable to cations Based on pharmacological and electrophysiological data these receptors have been classified into four main subtypes NMDA (*N*-methyl-D-aspartate), quisqualate, kainate and 2-amino-4-phosphonobutyrate[5] (APB) Recently, a G protein-coupled glutamate receptor representing a fifth class with a very different mechanism of action has been identified[6] The fact that the subtypes show differences in their distribution in the brain[7] indicates that they represent distinct gene products with unique structures and functions

Despite the prominent part these receptors play in normal synaptic transmission as well as in neuronal plasticity, their molecular characteristics have remained elusive, mainly because of a lack of ligands that bind irreversibly and with high specificity Conventional biochemical approaches to the isolation of these receptors have so far either not succeeded in progressing beyond crude receptor solubilization, as is the case for the NMDA[8] and quisqualate[9] subtypes, or have resulted in the isolation of ligand-binding proteins for kainate[10-12] or glutamate[13,14] whose functions remain unclear To circumvent these problems, we adopted a different approach involving the use of an oocyte expression system[15] to guide the selective cloning of cDNAs encoding functional glutamate-gated ion channels

Here, we report the isolation of a cDNA clone, GluR-K1, from a rat forebrain cDNA library This clone, on transcription *in vitro* and injection into *Xenopus* oocytes, expresses a functional glutamate receptor with the pharmacological properties of the kainate subtype The primary structure of the protein encoded by the GluR-K1 cDNA has been deduced, and it is compared with the sequences of other ligand-gated ion channels, the nicotinic acetylcholine receptors (nAChR), and the glycine and γ-aminobutyric acid ($GABA_A$) receptors The pattern of distribution of GluR-K1 mRNA is described and compared with kainate binding sites in the brain

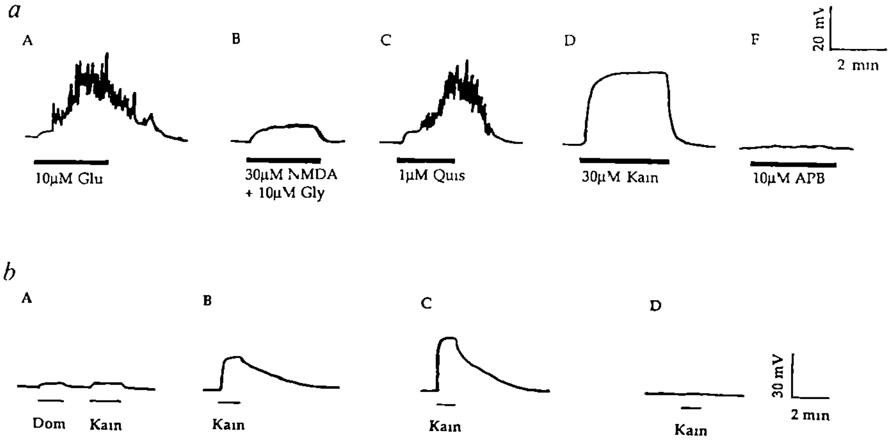


FIG 1 *a*, Voltage traces obtained from a single *Xenopus* oocyte injected with 75 ng poly(A)$^+$ RNA isolated from rat forebrain 2 days before recording Bars indicate duration of superfusion with indicated agonists Glu, L-glutamate, NMDA, *N*-methyl-D-aspartate, Gly, glycine, Quis, quisqualate, Kain, kainate, APB, 2-amino-4-phosphonobutyrate *b*, Voltage recordings from *Xenopus* oocytes 10 days (trace A) or 3 days (traces B–D) after injection with 25 ng λZAP RTB1 sense *in vitro* RNA (total library of 850,000 independent clones) (A), 25 ng λZAP-GluR-K1 sense *in vitro* RNA(B), 1 25 ng of pGluR-K1 sense *in vitro* RNA(C) or 1 25 ng of pGluR-K1 antisense *in vitro* RNA(D) Dom, domoate (10 μM), Kain, kainate (100 μM) Bars indicate duration of superfusion with agonist

METHODS RNA was isolated from rat total forebrain by the guanidine isothiocyanate method[39] and subsequently poly(A)$^+$ selected[40] The Uni-Zap™ XR cloning kit from Stratagene was used to construct a directional cDNA library of 850,000 independent clones in the bacteriophage expression vector λZAP II, where the insert clones are flanked by T3 and T7 RNA polymerase promoters at their 5′ and 3′ ends, respectively, allowing sense as well as antisense transcription[41] DNA from pools of phage clones was linearized with Xho I, and diguanosine triphosphate-capped RNA was synthesized *in vitro* using the RNA transcription kit from Stratagene A DNAse I digest was performed to remove the template after synthesis *Xenopus* oocytes were prepared as previously described[33] and injected with 1–100 ng RNA, usually in a volume of 50 nl Injected oocytes (20 per batch of RNA to be tested) were maintained in daily changed Barth's saline[33] at 17 °C During the initial stages of the library screening, oocytes were checked for responses after 3, 5, 7, 9 and 10 days During later stages of the screening, recordings were made on days 3 and 5 after injection To facilitate rapid, sensitive screening of large numbers of oocytes we used voltage recording rather than voltage clamp, as described previously[33] Depolarizations as small as 1–2 mV could be measured reliably

Isolation of clone λZAPII-GluR-K1

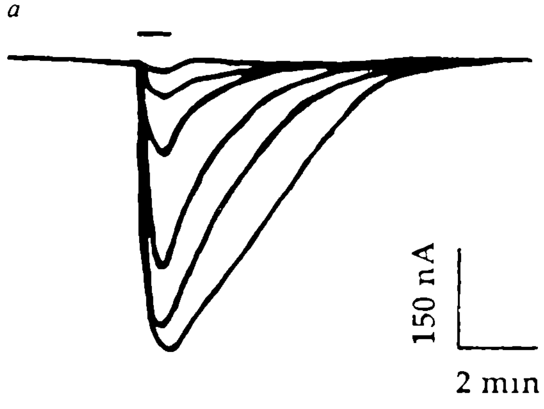
Xenopus oocytes were injected with poly(A)$^+$ RNA isolated from rat forebrain, 2-10 days after which the oocytes were tested electrophysiologically for their ability to respond to selective agonists for glutamate receptor subtypes Depolarizations induced by both glutamate and quisqualate displayed a biphasic pattern composed of a fast-acting, smooth, presumably ligand-gated, ion channel response and a longer lasting, fluctuating response that was probably second-messenger mediated[16] (Fig 1*a*, traces A, C) NMDA and kainate elicited smooth responses with fast onsets (Fig 1*a*, traces B, D) APB gave no response (Fig 1*a*, trace E)

A directional cDNA library (λZAPII RTB1, complexity, 8 × 10^5 elements) consisting of 18 independent sublibraries each of 44,000 clones was constructed from this poly(A)$^+$ RNA using the bacteriophage expression vector λZAPII A pool of *in vitro* transcripts made separately from all 18 amplified sublibraries was injected into oocytes Small depolarizations (1-3 mV) were seen in voltage recordings from oocytes challenged with 100 μM kainate 10 days after injection (Fig 1*b*, trace A) No responses to NMDA or quisqualate were detected Neither normal oocytes nor water-injected oocytes showed any response to glutamate receptor agonists Subsequently, pools of 44,000, 4,000, 400 and 40 clones were tested and in each of these tests at least one pool responded to kainate To ensure throughout the screening procedure that the very small responses observed initially were not recording artefacts, we showed that (1), responses in a given oocyte were reproducible, (2) responses were fast (within one second of agonist application), (3) responses were readily reversible on superfusion of the oocyte with control Ringer solution, and (4) domoate (10 μM) gave a response similar to the one elicited by 100 μM kainate The pools yielding the largest responses at each stage were selected for further subdivision The clones in the final positive pool of 40 clones were analysed for their insert size, and the 12 clones with the largest inserts (all >2 kilobases (kb)) were tested individually for their ability to direct the synthesis of a functional kainate receptor Only one clone, λZAPII-GluR-K1, carrying a 3-kb insert, was found to elicit kainate responses in oocytes (Fig 1*b*, trace B)

Characterization

The plasmid pGluR-K1 was rescued from bacteriophage λZAPII-GluR-K1 and, on transcription *in vitro*, sense RNA (but not antisense RNA) induced kainate responses when injected into oocytes (Fig 1*b*, traces C, D) As little as 10 pg of GluR-K1 sense transcript under voltage-clamp conditions (−70 mV holding potential) gave detectable responses to 100 μM kainate To rule out the possibility that GluR-K1 encoded a transcription factor, oocytes injected with pGluR-K1 *in vitro* transcripts were kept in medium supplemented with 50 μg ml^{-1} actinomycin D to inhibit endogenous transcription[17] These oocytes exhibited the same responses to kainate as those kept in control medium Therefore, injection-induced transcription from the oocyte genome seems unlikely to contribute to the observed responses

L-Glutamate evoked much smaller responses than did kainate, and even at 1 mM, elicited only 50% of the depolarization seen with 30 μM kainate This is consistent with previous reports that glutamate is only a weak agonist for the kainate receptor subtype[3] Other glutamate receptor agonists such as NMDA, quisqualate and L-aspartate evoked no responses when applied at 150 μM, 10 μM and 100 μM, respectively Unrelated neurotransmitter receptor agonists such as glycine, GABA, serotonin and nicotine also failed to evoke responses, even when tested at concentrations as high as 1 mM Glycine did not potentiate the kainate response (data not shown) Dose-response curves for kainate and domoate were recorded (Fig 2*b*), and EC_{50} (effector concentration for half-maximal response) values of



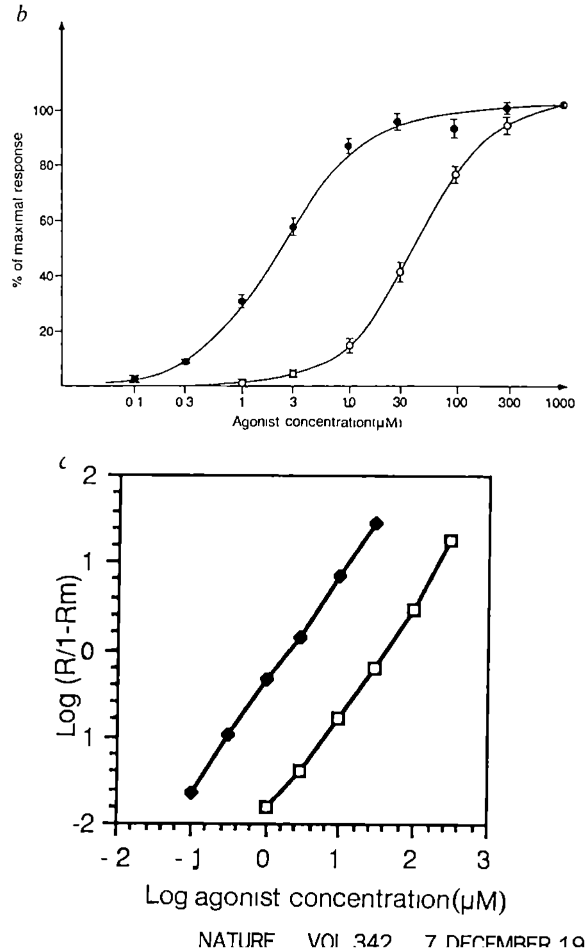
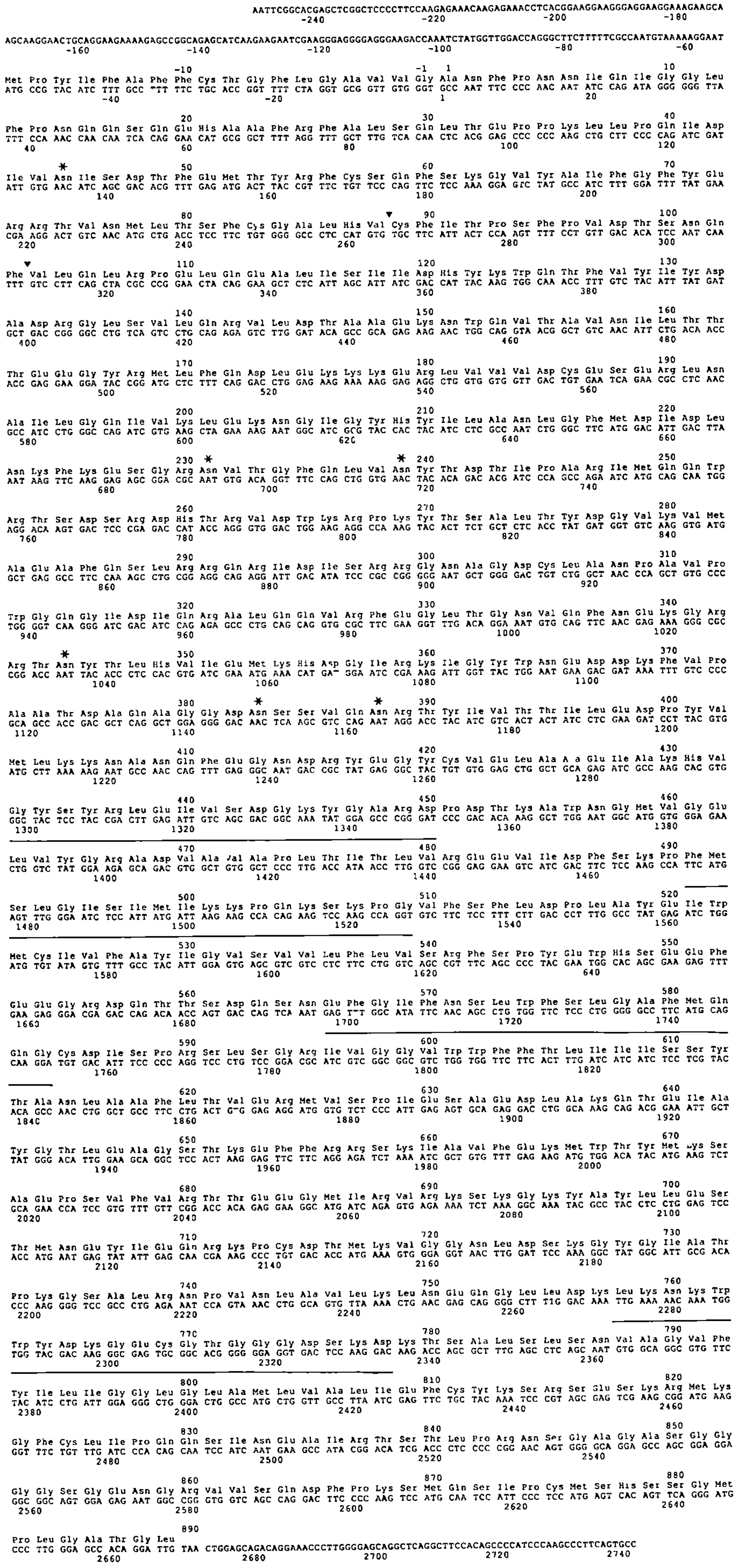


FIG 2 *a*, Superimposed voltage clamp traces, recorded at a holding potential of −70 mV from a single oocytes injected 2 days before recording The oocyte was injected with 1 25 ng pGluR-K1 *in vitro* synthesized RNA transcript, and challenged with increasing kainate concentrations of 1-1,000 μM The agonist was applied for 40 s, followed by control Ringer superfusion Intervals between applications were 5 min Note that the responses do not desensitize *b*, Dose-response curves for kainic acid and domoic acid, recorded as in *a*, each point represents the mean ±s e m of three responses, measured in different oocytes The EC_{50} values derived from these curves are 39 μM and 1 8 μM for kainic acid and domoic acid, respectively Closed circles, domoate, open circles, kainate *c*, Hill plots for oocyte responses to domoate (closed circles) and kainate (open circles), constructed using the mean values of the data from the dose-response curves of *b* Rm, maximal response, R, response Hill coefficients of 1 23 and 1 24 for kainate and domoate, respectively, were obtained



G 3 The nucleotide and deduced amino-acid equence of clone GluR-K1 Nucleotides are numered in the 5'- to 3'-terminal direction starting ith the first nucleotide of the codon for the utative amino-terminal residue of the mature rotein Cleavage of the assumed signal peptide ; predicted[22] at position 1 Nucleotides located pstream of the predicted 5'-terminal base have een assigned negative numbers Nucleotides −1) −54 encode a putative signal peptide[22], and ases −55 to −251 represent a 5'-untranslated egion At the 3'-terminus of the clone we found 1 nucleotides of untranslated sequence The educed amino-acid sequence for GluR-K1 is hown above the nucleotide sequence, the numering starting with the first residue of the mature rotein Possible extracellular *N*-glycosylation ites are marked with an asterisk above the aminocid residue The region marked by two arrows mino acids 89–103) bears some resemblance) the ligand-gated ion channel signature postuited by some workers[23,24] (see text and Fig 5*a*) he bars above the sequence mark the four proosed TMDs

IETHODS The insert cDNA of the bacteriophage lone λZAPII-GluR-K1 was cut out with *Eco* RI-*'ho* I, blunt-ended and subcloned into the *Sma* I-ite of the bacteriophage vector M13mp19 (ref 2), yielding clones with both orientations of the DNA Overlapping deleted subclones were contructed for each strand orientation using the yclone™ kit from USB Single-strand sequencing y the dideoxynucleotide chain-termination ıethod[43] was done with all (45) subclones, and 0 oligonucleotide primers were synthesized to acilitate sequencing across areas where comressions or gaps were encountered in the equences derived from the deletion subclones omplete sequences for both strands were ıus obtained IntelliGenetics software packages ntelliGenetics version 5 0, and PC/Gene™) were sed for analysing sequences

39 µM and 1 8 µM, respectively, were derived These values were in good agreement with results found in oocytes injected with total poly(A)$^+$ RNA[16 18] Hill coefficients calculated from these curves (Fig 2*c*) were 1 23 and 1 24 for kainate and domoate, respectively, indicating the possible cooperative action of several subunits The average reversal potential extrapolated from current responses to 10 µM kainate obtained at a series of holding potentials between 0 and −130 mV was 10 mV Responses to kainate did not desensitize, not even after prolonged (up to 10 min) superfusion with high concentrations (100 µM) of the agonist These findings are also consistent with earlier reports from experiments with kainate receptors expressed from total poly(A)$^+$ RNA[19] Similarly, the pharmacological profile of GluR-K1 (see Table 1), as revealed by the inhibiting properties of various known glutamate receptor antagonists, is consistent with previous reports for kainate receptors in systems where total poly(A)$^+$ RNA was used as a source of kainate receptor message[16,18] Of all the compounds tested (each at 1 mM), the glutamate receptor antagonist of broad specificity, kynurenic acid, was the most potent inhibitor of the kainate-evoked depolarizations in oocytes injected with GluR-K1 RNA synthesized *in vitro* To a lesser extent, γ-D-glutamylglycine (γ-DGG), reported to block preferentially kainate and NMDA receptors but not quisqualate receptors[20], inhibited kainate responses, as did γ-D-glutamylaminomethylsulphonate (GAMS), which acts preferentially on kainate and quisqualate receptors[21] Similarly, 2,3-*cis*-piperidine dicarboxylic acid (PDA), which is known to block all subtypes of glutamate receptors[5], blocked the GluR-K1 response Glutamate diethylester (GDEE), which is thought to inhibit preferentially quisqualate-type glutamate receptors[5], did not block the response significantly, but instead displayed weak agonist properties The NMDA receptor antagonists AP5 (D-(−)-2-amino-5-phosphonovaleric acid) and CPP (3-(2-carboxypiperazin-4-yl)-propyl-1-phosphate), as well as NMDA itself, slightly inhibited the kainate responses, thus acting as weak antagonists without showing any agonist properties

Taken together, the electrophysiological properties observed in oocytes injected with GluR-K1 transcript, as well as the observed pharmacological properties, strongly indicate that GluR-K1 represents a functional kainate receptor indistinguishable from the one observed in oocytes injected with total poly(A)$^+$ RNA Thus, it appears that the single protein subunit encoded by GluR-K1 is sufficient to form an active receptor–ion-channel complex Whether this complex is composed of a single protein subunit *in vivo*, or whether it is a homo-oligomer assembled from several identical subunits, cannot be decided Given the fact that the depolarization induced by RNA *in vitro* transcripts of the isolated clone GluR-K1 is smaller than the one observed with forebrain poly(A)$^+$ RNA (Fig 1), it seems possible that additional receptor subunits exist that mig modify the channel properties The slight deviation of th Hill coefficient from unity suggests the presence of interactin multiple subunits

TABLE 1 Pharmacology of the glutamate receptor encoded by GluR-K

Compound tested	Compound alone (%)*	Compound plus kainate (%)†
Kainate (agonist control)	100	100
Kynurenic acid	−3 4±0 2	9 6±1 3
γ-DGG	−0 1±0 5	30 8±1 1
GAMS	1 0±0 6	30 7±1 1
GDEE	24 8±5 7	97 8±6 6
PDA	2 5±0 7	31 3±2 0
APV	3 1±1 4	73 7±3 2
CPP	9 1±0 7	78 8±0 9

Oocytes had been injected with 1 25 ng GluR-K1 *in vitro* sense RNA days before the recording The oocytes were voltage-clamped at −70 m and the test compounds (all at 1 mM, except kainate, which was 30 µ applied by rapid superfusion, with 5-min intervals between drugs Pe currents were recorded, and each number represents the average of recordings from 3 different oocytes, ±s e m The 100% current respon corresponds to 40–200 nA, depending on the oocyte

* Per cent of the response evoked by 30 µM kainate immediate before dr application

† Per cent of the response seen with 30 µM kainate alone immediat before application of drug–kainate mixture

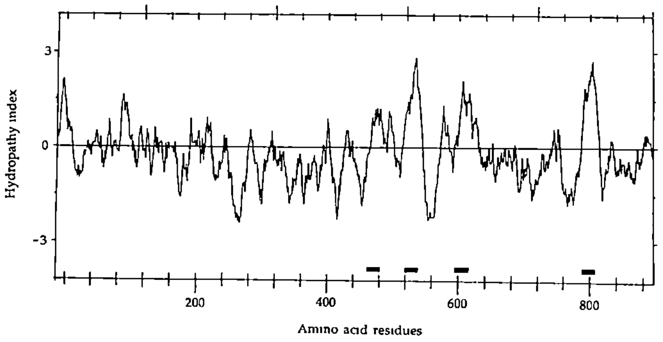


FIG 4 Hydropathy plot of GluR-K1 (residues +1 to 889), using the algorithm of ref 44 with a window setting of 15 The calculation was done with the University of Wisconsin software package[45] The bars mark the regions of the four proposed TMDs

DNA and deduced amino-acid sequences

The cDNA insert of the plasmid pGluR-K1 was subcloned int M13mp19 and sequenced (Fig 3) An open reading frame 2,721 base pairs (bp) was found within the total length 2,992 bp Thus, the predicted protein consists of 889 amin acids, with a calculated M_r of 99,769 The deduced protei sequence contains a putative signal peptide of 18 amino aci at its N terminus, with a predicted cleavage site that confor to empirical rules[22] The N terminus of the protein therefore expected to be located extracellularly There are six possibl *N*-glycosylation sites present in the presumed extracellul domain Sequence comparisons with other ligand-gated io channels, the nicotinic acetylcholine receptors, GABA$_A$ rece tors and the glycine receptor, reveal little overall homology an much less than anticipated in the light of the recently propose 'superfamily' hypothesis[23,24] of ligand-gated ion channels I fact, using the sequence comparison algorithm of Feng, Johnso and Doolittle[25], no significant over-all homology is detecte between the protein encoded by GluR-K1 and the neuron neuronal nAChR subunits[26,27] $\alpha2, \alpha3, \alpha4, \beta2, \beta3$ or β GABA$_A$ receptor subunits[28] α or β, or the glycine recept subunit of M_r 48K[29]

All ligand-gated ion-channel subunits sequenced previousl have a conserved extracellular region characterized by two cy teine residues spaced 14 amino acids apart, with conserve proline and aspartate residues located 8 and 10 amino acid respectively, downstream from the first of these two cysteine This hypothetical signature for neurotransmitter recepto channel complexes[24] is conserved only poorly in the prote encoded by GluR-K1 Although the proline and aspartat residues are present, the first cysteine residue is located only residues upstream from the proline residue, and the secon cysteine residue is absent (Fig 5*a*)

A hydropathy plot analysis of GluR-K1 (Fig 4) reveals severa regions that are candidates for transmembrane domain (TMDs) The region between amino acids 455 and 810 is o particular interest as its hydropathy profile resembles that see in the other ligand-gated ion channels three closely space putative TMDs which are separated by ~175 amino-aci residues from a fourth putative TMD which is located close t the C terminus of the protein Although the presumed TMD I is very prominent, the assignment of three TMDs in the 'TMD cluster' region (amino acids 521-612) is less obvious Clearly, there are two very good candidate regions for TMDs, located at amino-acid residues 521-539 and 596-614, respectively A third TMD might be assigned to the region between amino-acid residues 463 and 480, or alternatively between residues 567 and 585 We favour the interpretation that the region between residues 463 and 480 actually represents a third TMD (see below) Thus, we would assign the following four transmembrane regions in GluR-K1 TMD I, located between amino-acid residues 463 and 480, TMD II between residues 521 and 539, TMD III between residues 596 and 614, and TMD IV between residues 788 and 808 The region between residues 463 and 480 is proposed as TMD I for two reasons First, in all ligand-gated ion channels the putative TMD I contains a conserved proline residue[23 24] which is located at position 12 in all nAChRs, and at position 8 in the GABA and glycine receptors In the putative TMD I of GluR-K1, a proline is found at position 12 Second, putative TMD II in GluR-K1 shows sequence homology with putative TMD II regions in the nAChRs (Fig 5*b*) Homology in this region might be expected in view of the fact that both nicotine-gated as well as glutamate-gated ion channels have to provide the environment for the passage of cations, and TMD II is the region that is thought to line the ion channel of the nAChR[30] Taken together, the structural features found in the GluR-K1 protein are consistent with this receptor being a member of the superfamily of ligand-gated ion channels The homologies detected, however, are not strong enough for an unequivocal conclusion to be reached, this will require the analysis of the primary structures of more members of the glutamate receptor family

Regional tissue distribution of GluR-K1 RNA

Northern blot analysis with the full-length GluR-K1 cDNA probe (Fig 6) revealed the presence of large amounts of a RNA species, 5 2 kb in size, in poly(A)$^+$ RNA isolated from cortex, cerebellum and hippocampus, and trace amounts of RNA in the brainstem Additionally, two minor bands corresponding to RNAs of sizes ~3 9 and ~3 2 kb were seen in these tissues These minor bands were also observed under conditions of high-stringency hybridization (see legend to Fig 6), ruling out the possibility of cross-hybridization to distantly related mRNAs Most probably, these minor bands represent GluR-K1 RNA transcripts emanating from different polyadenylation sites Control tissue from outside the CNS (liver and lung) showed no traces of GluR-K1 RNA, and neither did poly(A)$^-$ RNA from any of the tissues tested The regional distribution of GluR-K1 RNA in the brain correlates well with autoradiographic studies of kainate-binding sites, which showed the highest density to be in the hippocampus, with lesser density in cortex and cerebellum[7,31] and undetectable labelling in brainstem

Discussion

We have isolated a cDNA clone, GluR-K1, from a rat forebrain cDNA library by screening for expression of kainate-gated ion channels in *Xenopus* oocytes This cDNA encodes a functional glutamate receptor consisting of a single protein of calculated M_r 99,769, disregarding any possible post-translational modifications This protein forms an ion channel which possesses electrophysiological and pharmacological properties consistent with those reported for kainate receptors studied in more complex systems[16,18] or cultured neurons[32] The size of

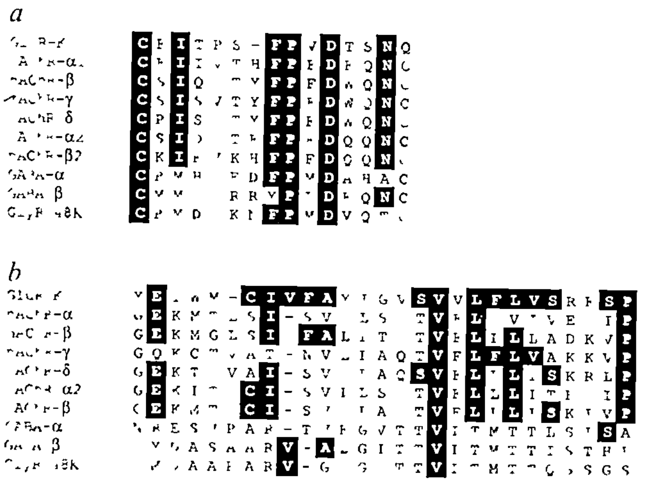


FIG 5 *a*, Comparison of the extracellular region located between amino-acid residues 89 and 106 of GluR-K1 with the 'Cys-Cys-loop' region found in all other ligand-gated ion channels, showing sequence homology Sequences of nAChR subunits α, $\beta 1$, γ, δ are from mouse muscle[46], those of nAChR subunits $\alpha 2$ and $\beta 2$ are from rat brain[26] GABA$_A$ subunits α and β are from calf brain[24] GlyR 48K is the 48K subunit of the glycine receptor from rat brain[29] Boxed amino acid residues are found at identical positions in GluR-K1 as well as in at least one of the other receptor sequences One gap has been introduced arbitrarily *b*, Comparison of the putative TMD II region of Glu-R-K1 with hypothetical TMD II regions of other ligand-gated ion channels[24,26 29 46] (as *a*), suggesting protein-sequence conservation

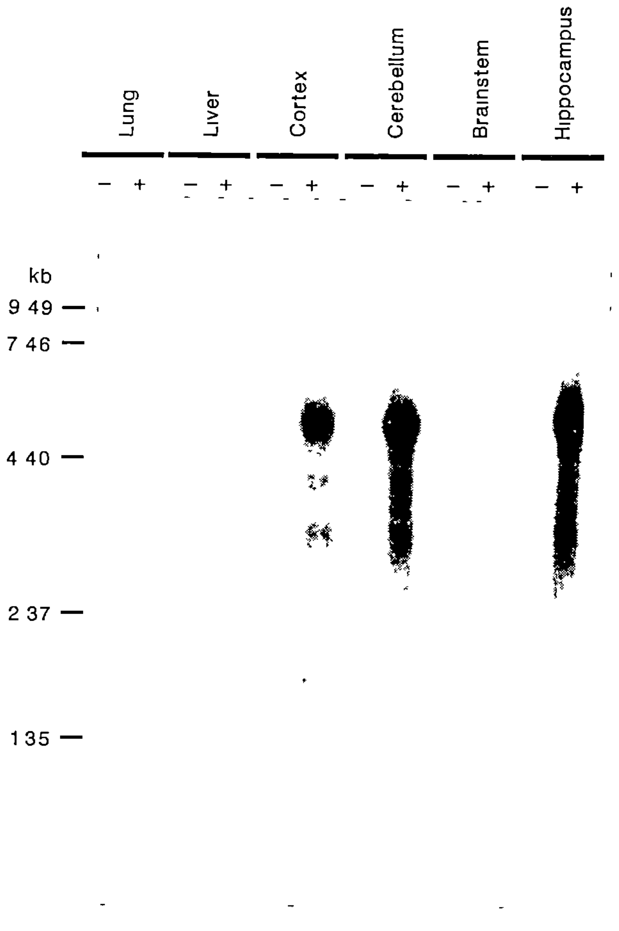


FIG 6 Northern blot analysis of six rat tissues for the presence of GluR-K1 message Poly(A)$^+$ RNA (3 μg per lane) and poly(A)$^-$ RNA (6 μg per lane) were prepared as in Fig 1, separated on 1% formaldehyde-agarose gels and transferred to a Nytran filter A full-length GluR-K1 DNA probe was prepared by the random prime method using the Amersham Multiprime DNA labelling kit and was hybridized (10^6 c p m ml^{-1}) to the filters Prehybridization and hybridization were at 42 °C in 4x SSC/40% formamide, and filters were washed in 0 2×SSC/0 1% SDS at 55 °C, followed by 0 1× SSC/0 1% SDS at 65 °C The RNA ladder from BRL was used to standardize RNA sizes + and − Poly(A)$^+$ RNA and poly(A)$^-$ RNA, respectively

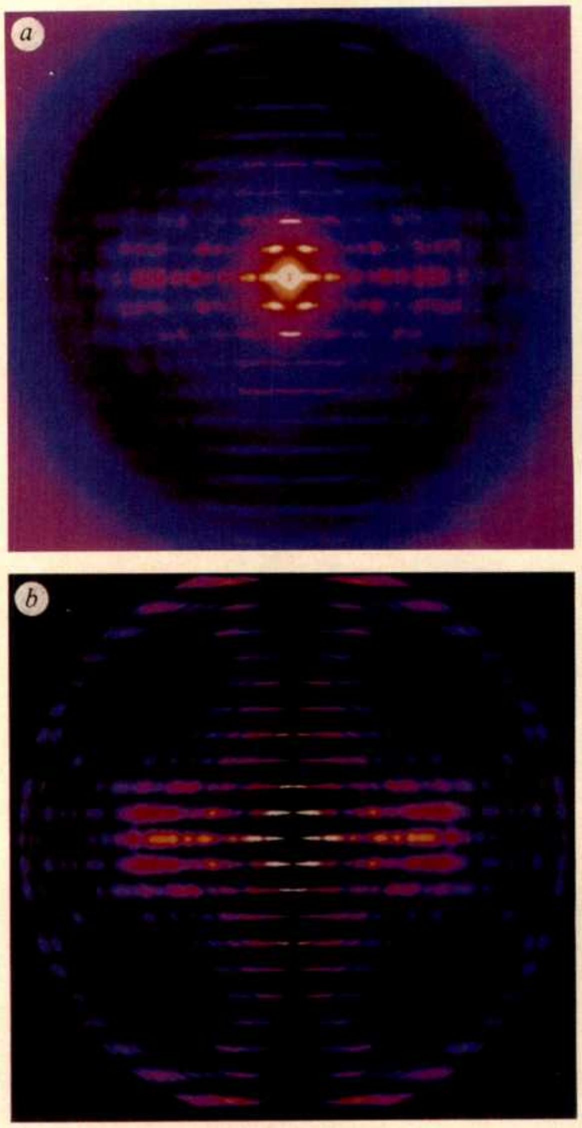


FIG. 1 X-ray diffraction pattern from reconstituted L-type straight flagellar filaments. *a*, Observed diffraction pattern. Straight-type flagellar filaments were isolated from *S. typhimurium* strain SJW1660 (provided by Dr R. Kamiya, Nagoya University) as previously described[24]. The filaments were depolymerized into monomers and were purified by fast protein liquid chromatography (FPLC) on a Mono-Q column (Pharmacia). The monomers were repolymerized into the filaments with the desired length distribution by adding 1 M ammonium sulphate to a flagellin solution (10 mg protein ml^{-1}). The reconstituted filaments were washed in 0.2 M glycine–NaOH, pH8, and were centrifuged for 20 h at 10,000*g*. The resulting soft pellet was transferred into a capillary tube. The filaments were orientated by being forced to flow back and forth in the capillary for 15 min to 2 h. The protein concentration of the specimen was 100–150 mg ml^{-1}. We used reconstituted filaments because native filaments are ~10 μm long and therefore the resulting pellet is too hard to orientate. The filaments were reconstituted from flagellin monomers so that the length distribution could be controlled. Well-oriented specimens were obtained with filaments of an average length of ~0.5 μm. Specimen preparation will be described in more detail elsewhere (I.Y., F.V. T. Noguchi and K.N., manuscript in preparation). Fibre diffraction experiments were done with a RIGAKU RU200 rotating anode X-ray generator using a 0.1-mm focal cup, operated at 40 kV and 25 mA and double mirror optics. CuKα-radiation (λ = 1.5418 Å) was used. The diffraction patterns were recorded on a photostimulable phosphor-plate (Imaging Plate, Fuji Film) with a 20-h exposure and a specimen-to-film distance of 170 mm. Recorded images were scanned with a Fuji Film BA100 Imaging Plate scanner using 0.1 mm rasters and were displayed on a Seiko D-SCAN GR4416 graphics terminal. Photographic copies were made with a Dunn Instruments MultiColor type 638 colour hardcopy camera. The much higher sensitivity and wider dynamic range of the Imaging Plate compared with those of photographic films[33] made the search for well-oriented specimens efficient and made it possible to record diffraction patterns with high signal-to-noise ratios in a relatively short time. *b*, Simulated diffraction pattern. The layer-line intensities were extracted from the diffraction pattern shown in *a* with the angular deconvolution method[25,26] and the diffraction pattern was reconstructed using these intensities and the angular spread function without background.

image reconstructions from electron micrographs have shown the subunit shape on the surface of the filament[12,13]. It has not yet been possible, however, to visualize the subunit packing in the filament to explore the architecture of this assembly. Also, flagellar growth *in vivo* is known to occur at the distal end of the filament[14,15], where the HAP2 protein forms a cap structure, which presumably serves to prevent arriving flagellin subunits from diffusing away before polymerizing[16,17]. It is thought that the flagellin molecules are transported through the central channel of the filament, but the channel structure has not yet been unambiguously determined.

The helical form of the wild-type filament is postulated to involve two different conformations of flagellin monomers in one filament[5,18–21]. The structural symmetry, therefore, is complicated and analysis of wild-type filaments is not feasible. Straight filaments, in which all subunits are in the same conformation, would make structural analysis possible. Such filaments have been isolated from mutants of *S. typhimurium*[22,23].

We have solved the structure of a straight-type mutant bacterial flagellum at a resolution of 20 Å, using X-ray fibre diffraction. The electron density map clearly shows the shape of the whole subunit as well as the packing and indicates that the innermost domain of each subunit is mostly responsible for filament formation. The map also shows the central channel of the filament, which is large enough for folded flagellin monomers to pass through. Based on other evidence, we have made tentative assignments of primary sequence to the morphological domains observed. In particular, because a proteolytic fragment of flagellin missing portions of the terminal regions cannot polymerize into the filament[24], the innermost domain was assigned to the amino- and carboxy-terminal segments. The intensity distribution at 10-Å resolution in the diffraction pattern indicates that the innermost domain, which forms the core of the filament, consists of a bundle of α-helices aligned in parallel to the filament axis.

X-ray fibre diffraction

There are two types of straight filament (L- and R-type), whose subunits are assembled with opposite-handed helical symmetry. Figure 1*a* shows a fibre diffraction pattern from reconstituted L-type straight filaments from *S. typhimurium* strain SJW1660. Flagellin from this strain differs from that of the wild-type strain SJW1103 by only one amino acid (S. Kanto and S.-I. Aizawa, personal communication). Because the diffraction pattern shows very little interference from lateral lattice formation, it is equivalent to the cylindrically averaged diffraction pattern from a single filament. The helical symmetry is reasonably well approximated by 331 subunits in 60 turns of the basic helix. The pitch of the basic helix is 26.35 Å, as measured from layer lines 2, 4 and 6, where the Bessel orders 1, 2 and 3 contribute, respectively. The axial rise per subunit is 4.78 Å, as measured from layer line 11, where the Bessel order 0 contributes. The structure repeats every 1,581 Å along the filament. Layer lines indexed by 1,581 Å spacing are not individually visible because

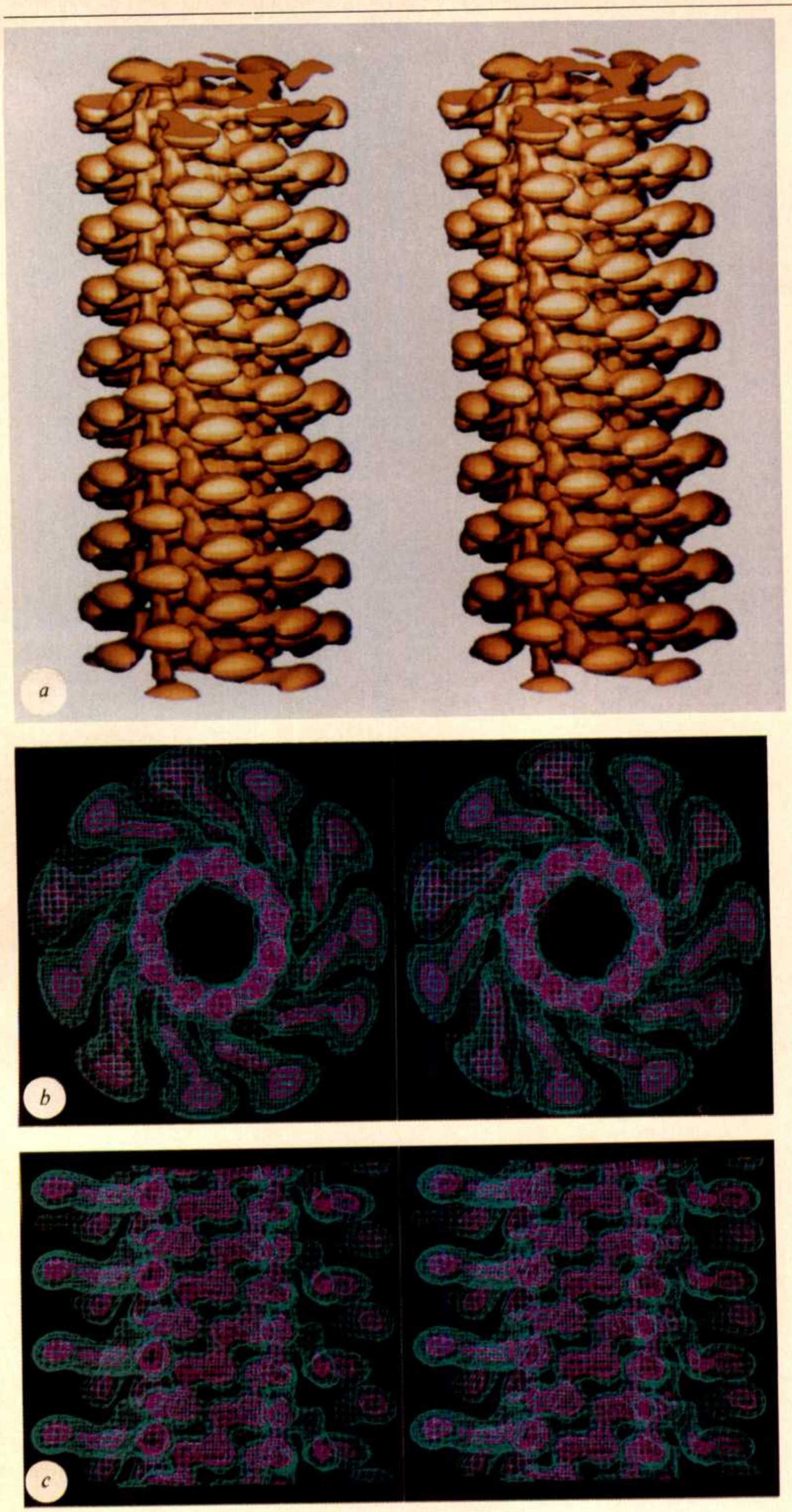


FIG. 5 Refined electron density maps. *a*, Oblique view of a shaded solid representation of the contoured map. The map is contoured at level 30, which gives ~80% of the correct volume. The length of the map along filament axis is 500 Å. *b*, Cross-section of the filament with 52.5 Å axial thickness. *c*, Longitudinal section. The section is 55 Å thick and 200 Å long axially. The position of this section is indicated on the left side of *a*. The maps in *b* and *c* are both contoured at two levels of 30 and 90.

Figure 5*c* is a longitudinal section of the filament viewed from inside, being 55-Å thick and 200-Å long along the axis; the region comprising this section is indicated on the left side of Fig. 5*b*. Axial stagger of the subunits is visible in the middle of the section shown in Fig. 5*c*. Neighbouring subunit relations are along the 5-start left-handed helix to the top right, along the 6-start right-handed helix to the top left, and along the basic helix nearly horizontally as indicated in Fig. 2*a*. The domain contacts are so close in the core that it is hard to distinguish from the lower contour whether the vertical rod density lying along the 11-start helix or the horizontal density along the basic helix belongs to a single subunit. From the higher contour the latter is more likely to belong to a single subunit. Then, the subunit contacts are extensive along the basic and the 5-start helices, and less extensive along the 6-start helix.

A subunit can be divided into three domains, which are labelled in Fig. 6*a*. Measuring along the filament radius, domains 1, 2 and 3 extend from 30–55 Å, 55–80 Å and 80–120 Å, respectively. The innermost domain (domain 1) has the highest density and is responsible for most of the filament formation. The middle domain (domain 2) seems to have some interaction with domain 3 of the neighbouring subunit in the clockwise direction, but this interaction is not as close as that between domains 1 of neighbouring subunits. Domain 3 is on the filament surface and is presumed to determine the antigenic properties. Domain 1 seems to consist of two globular subdomains, only one of which is extensively connected with domain 2 within a subunit. Domain 2 is a vertical rod of 35-Å length, slightly tilted towards the filament axis. Domain 3 appears somewhat fin-shaped, 50 Å long and 50 Å wide at its widest part with an axial thickness of ~25 Å.

Assignment of amino-acid sequence

The volume of each domain was estimated from the refined map and the corresponding molecular weights are listed in Table 1. The distribution of α-helical secondary structure in flagellin was predicted from the primary sequence and is plotted in Fig. 6*b*, with the domain boundary positions determined by proteolytic digestion[24]. F40 and F27 represent fragments with M_rs of 40K and 25K, respectively, which were isolated from a digestion mixture. The predicted secondary structure is consistent with circular dichroic spectra of flagellin and its fragments in the far ultraviolet region (F. V., H. Uedaira, S. Kidokoro and K.N., manuscript in preparation). It has also been shown that the terminal regions of monomeric flagellin are partially unfolded and flexible[24].

Because the terminal regions of flagellins of different serotype or even from different species have high amino-acid homology, these terminal regions are believed to be important in filament formation[4,29,30]. Indeed, the F40 fragment, which is missing the 65 N-terminal residues and 44 C-terminal residues of flagellin, cannot form filaments even under conditions that favour polymerization[24]. It is reasonable, therefore, to assign these terminal regions to domain 1, the estimated volume of which is consistent with such an assignment. As the F27 fragment contains the region of highly variable sequence responsible for flagellar antigenicity[31], it probably corresponds to domain 3 located on the filament surface; the estimated volume of domain 3 is consistent with this. Domain 2 can, therefore, be assigned to the parts of F40 absent from F27 on the basis of both deduction and estimated volume. These assignments are summarized in Fig. 6. A part of D2 indicated in Fig. 6*b* could, however, be contributing to the filament core, because the amino-acid replacements which

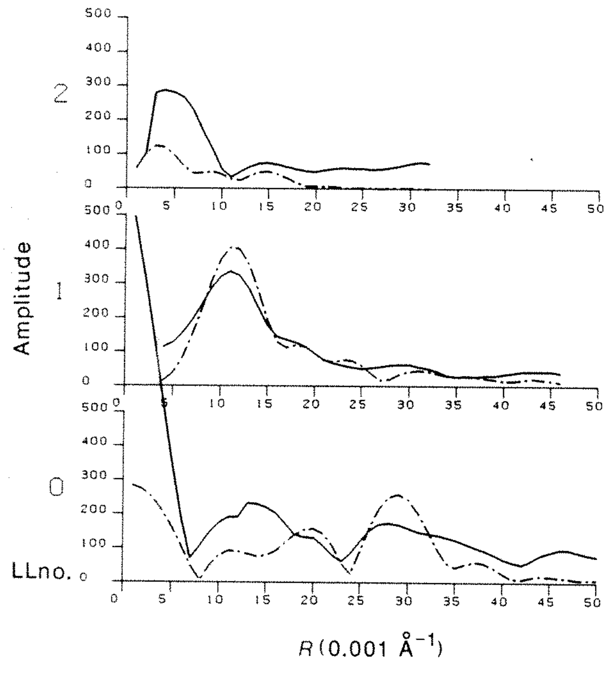


FIG. 3 A comparison of layer-line amplitudes. Solid line, X-ray fibre diffraction data; dashed line, data used in three-dimensional image reconstruction from electron micrographs[13]. The data represented by the dashed line were calculated by grouping layer-line intensities from the original data[13] according to the *n,l* plot in Fig. 2*b*. It was not possible to obtain the data for the peak at the origin of layer line 0 from the X-ray diffraction pattern because of the interfilament-interference effect observed here. Therefore, the data from the image reconstruction work[13] were scaled-up three times so that the initially negative radial density distribution at the filament axis became zero. Adjusting the negative density level to zero improves the molecular envelope, resulting in the better-refined images. LL no., Layer-line number.

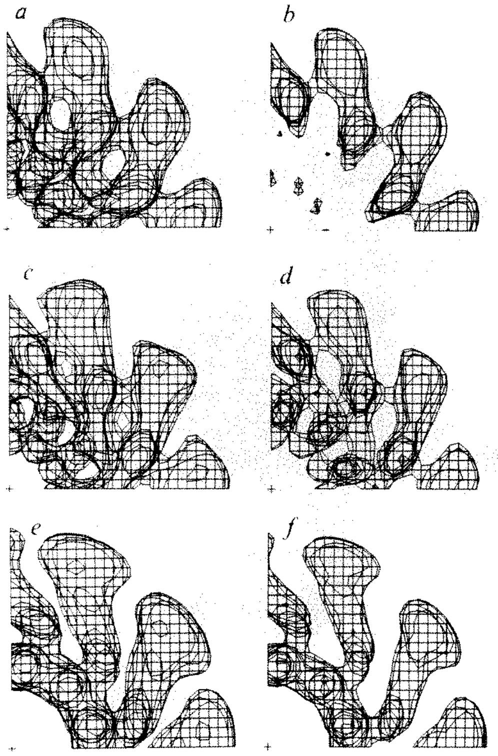


FIG. 4 Improvement of the electron density maps during the phase refinement procedure. Only a quarter of a cross-section of the filament is shown for each map. The sections are 52.5 Å thick. *a*, *b*, Three-dimensional image reconstruction from electron micrographs of frozen hydrated specimens[13]. *c*, *d*, Maps calculated with the amplitudes from the X-ray diffraction pattern and the phases from the three-dimensional image reconstruction. *e*, *f*, Maps calculated after 10 cycles of the solvent-flattening phase refinement procedure. The maps are contoured at density level 30 in *a*, *c* and *e*, and 50 in *b*, *d* and *f*, where the highest density is ~150 in arbitrary units.

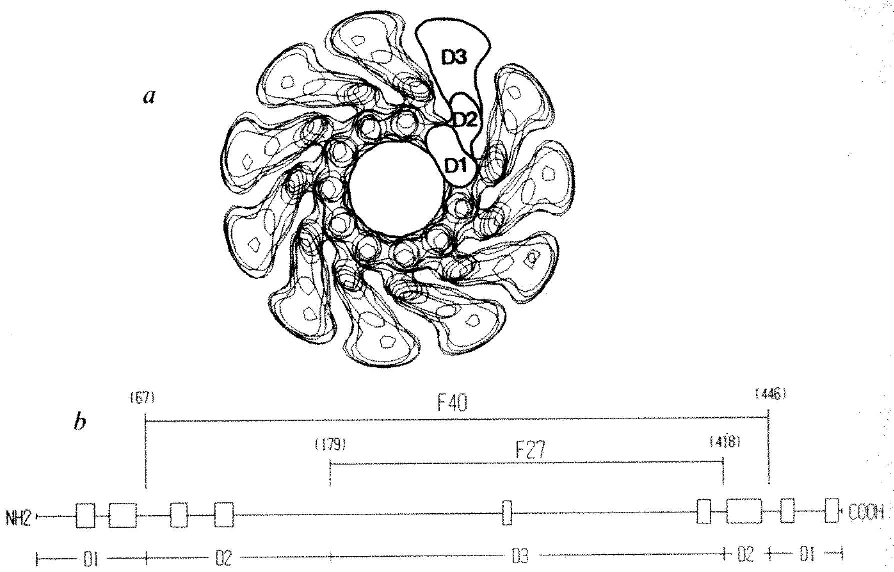


FIG. 6 Amino-acid sequence assignment for domains in the electron density map. *a*, Contoured electron density map viewed along the filament axis. Twelve sections at 4.7-Å intervals are superimposed and the domains are labelled. *b*, Diagram showing the predicted distribution of α-helices and the domain boundary positions. Rectangles represent α-helices. The secondary structure was predicted using the program ALB[35] obtained from Brookhaven Protein Data Bank. The domain boundary positions were determined by proteolytic digestion[24].

affect filament shape are in the D2 region and the interspecific sequence homology of this region is as high as that of D1[4,29,30]. Thus, the sequences with high α-helical content are assigned to domain 1 and 2 and the non-α-helical part to domain 3; this is consistent with the interpretation of the intensity distribution at ~10-Å resolution in the diffraction pattern. Preliminary model fitting shows that the cross-section of domain 1 shown in Fig. 6*a* is just the right size to consist of two bundles, each containing four α-helices, arranged side by side with their axes parallel to the filament axis. Differential scanning calorimetry of F27 suggests that domain 3 contains two thermodynamically independent units (F.V., H. Uedaira, S. Kidokoro and K.N., manuscript in preparation), although it is not visible in the map at the present resolution.

Central channel

Although it is known that the growth of flagellar filaments *in vivo* occurs at the distal end[14,15], it is not known how flagellin monomers reach the tip of the filament. Although the best explanation is that flagellin monomers pass through the filament's central channel, there has not been any experimental evidence showing that the central hole is large enough to accomodate folded flagellin molecules. Unlike tobacco mosaic virus[32], electron micrographs of negatively stained filaments do not show the hollow channel, for reasons that are unknown. Three-dimensional image reconstructions from electron micrographs[13] show a small hole (Fig. 4*a*), which could suggest that flagellin monomers are transported in an unfolded and stretched conformation. But the density distribution of the core-part forming the hole was weak and noisy. The refined electron density map using X-ray fibre diffraction data shows well-defined subunit packing in the core region and a central hole of ~60-Å diameter, which is large enough to accommodate the folded flagellin molecule with the elongated shape shown in Fig. 6*a*. It is probable, therefore, that flagellin molecules are transported through the central channel with the same conformation as they have in solution. A hole of this size should be visible by electron microscopy. Electron micrographs of transverse thin sections of reconstituted filaments show a large central hole, supporting the result described above, whereas native filaments have a filled appearance, indicating that the central hole of native filaments is filled with flagellin molecules (T. Akiba, C. J. Jones and K.N., manuscript in preparation).

Implications for flagellar function

Having made the correspondence, shown in Fig. 6, between the volumes of the domains that appear as distinct entities in Fig. 5 and fragments of flagellin defined in biochemical assays, it is easy to understand how each domain of the flagellin subunit can be independently responsible for its respective role. Antigenicity is an important function of the highly variable domain 3; this variability can leave the filament structure undisturbed, because domain 1 alone seems to be able to form the filament backbone regardless of the conformation of domain 3, provided it is not oversized. If the boundary of domain 1 shown in Fig. 6*a* is correct, subunit addition during polymerization proceeds along the basic helix at the bottom of the filament shown in Fig. 5*a*, where only one site would be available for subunit addition at a time. About 100 residues at the terminal regions of flagellin, which are in a flexible conformation in solution (ref. 24; and S.-I. Aizawa, F.V., R. Ishima and K. Akasaka, manuscript in preparation), become well folded in an α-helical conformation when bound to the end of the basic helix. Because it is difficult to conceive how these terminal regions could be recognized by the binding site if they were totally unfolded, they must be in a somewhat folded conformation. In agreement with this, circular dichroic spectra indicate the presence of some unstable α-helical structure in these regions of monomeric flagellin[24].

The model proposed for flagellar polymorphism[5,18-21] assumes high cooperativity within a protofilament running along an 11-start helix, and independence between protofilaments. The structure presented here does not directly support this model, because one of the two subunit-contacts along a protofilament is relatively weak and a subunit extends over two protofilaments. But the structure presented here is not inconsistent with the model either, because high cooperativity does not necessarily require a direct connection along the protofilament, and a protofilament consisting of two subdomains of domain 1, each coming from neighbouring subunits, could be independent from neighbouring protofilaments, to which the other subdomains of domain 1 contribute, if subdomains behave as independent subunits. In fact the present structure, in which a subunit appears to extend over two protofilaments, seems to correspond to the scheme proposed by Calladine[19,20]. Because the resolution of the structure at present is not high enough to permit the confident description of the subunit connections, further attempts to reconcile it with the current model of polymorphism are premature. Comparison of this structure with that of R-type straight filament should help resolve this question. ☐

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1. Berg, H. C. & Anderson, R. A. *Nature* **245**, 380–382 (1973).
2. Silverman, M. & Simon, M. *Nature* **249**, 73–74 (1974).
3. Larsen, S. H., Reader, R. W., Kort, E. N., Tso, W. W. & Adler, J. *Nature* **249**, 74–77 (1974).
4. Joys, T. M. *J. biol. Chem.* **260**, 15758–15761 (1985).

If the upstream flow speed becomes relativistic ($V_1 \leq c$), anisotropies in the particle distribution at the shock invalidate the diffusion approach (see Fig. 1). Numerical simulation, on the other hand, shows that $\Delta p/p \gg 1$, with a resulting enhancement in the acceleration rate.

For $V_1 = 0.96c$, $V_2 = 0.32c$ (that is, for a relativistic compression ratio r of 3, see ref. 7), Fig. 2 shows the frequency distribution of $\Delta p/p$, which averages at ≈ 10.5. The large resultant fractional energy increase is, on average, approximately γ^2, where $\gamma = (1 - V^2/c^2)^{-1/2}$, and V is the relativistic difference between V_1 and V_2. The average relativistic cycle time t_r, for a given initial momentum p_i, is also provided by the model. Typically, within a time t_r, a cosmic-ray particle in the shock vicinity crosses it twice, and is accelerated from momentum p_i to momentum $p_f = (1 + \Delta p/p)p_i \approx 11.5p_i$. To compare the relativistic result with diffusion theory, the nonrelativistic acceleration time, t_{nr} given by equation (2), is calculated using identical values of p_i, p_f, V and λ/p. It is found that $t_{nr}/t_r \approx 13.5$.

Figure 3 shows how an escape probability per cycle P can be deduced from a frequency histogram giving the number of cycles before escape. Because neither this, nor $\Delta p/p$ are small compared with unity, the particle number power-law index of $-1 - (P/(\Delta p/p))$ does not develop in the initial few decades of energy beyond the injection energy.

Relativistic shocks have been studied by Bogdan and Webb[8], and by Kirk and Schneider[9]. The former used a two-stream approximation, wherein particle velocity directions are reversed in each scattering. Their results are more dramatic than ours. The latter, on the other hand, used pitch-angle diffusion with a small mean scattering angle, and reported considerably milder acceleration. Owing to the breakdown of diffusion theory in relativistic shocks, a sequence of many small-angle scatterers can no longer be replaced by a few large-angle scatterers with longer mean free path. Because our results correspond to isotropic scattering, they fall somewhere in between those of these two previous works.

To support our use of isotropic scattering centres, we develop an argument based on scaling known properties of energetic-particle scattering in the interplanetary medium. Cosmic-ray modulation studies indicate that $\lambda \propto L_m$ (magnetic rigidity) in the relativistic regime[10]. Detailed trajectory integration in a field model based on the region around an interplanetary shock reveal large pitch-angle changes ($\Delta \cos\theta \sim 0.5$–$1$) occurring in times that are short compared with the travel time between these events (see Figs 2a, b, c of ref. 11). The mean free path λ derived for this particular data set can be fitted by $\lambda = K\rho_c$ for a particle cyclotron radius ρ_c with the empirical constant $K = 41$ (ref. 12). For $L_m = 200$ MV we have $\lambda = 0.03$ AU which is roughly the separation seen between the large-amplitude waves existing behind the shock wave we are studying (see Figs 1a, b of ref. 13). Quasilinear scattering theory, based on a series of small pitch-angle changes, certainly does not apply in the disturbed ($\Delta B/B \approx 0.3$) interplanetary field[10], and we suggest that such knowledge as we have of AGN jets and shocks indicates a similar regime.

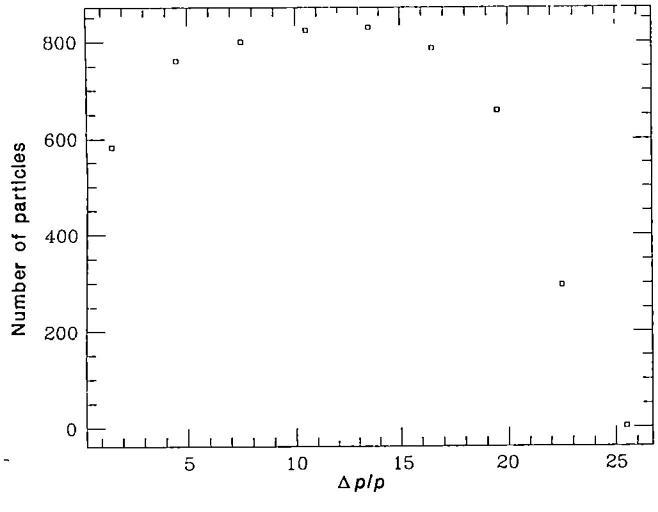

FIG. 2 Frequency distribution of $\Delta p/p$, the fractional change of cosmic-ray momentum within a cycle of downstream–upstream–downstream traversals, for a parallel relativistic shock with $V_1 = 0.96c$, $V_2 = 0.32c$.

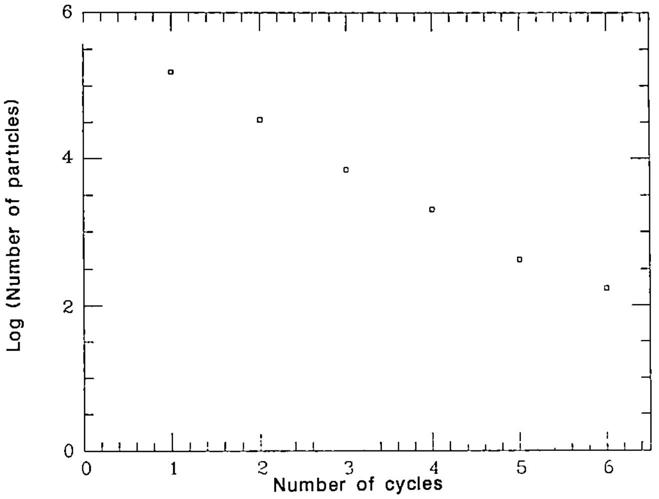

FIG. 3 Frequency distribution of n, the number of downstream–upstream–downstream cycles that a cosmic-ray particle makes before it leaves the shock, for a parallel shock with $V_1 = 0.96c$, $V_2 = 0.32c$. Note that the frequency is proportional to $(1 - \alpha)^n$, where α is the escape probability per cycle. As the frequency is given on a logarithmic scale, the plot yields an approximate straight line of slope $-\alpha$.

Magnetic fields in AGN hotspots, the shock working surface of our model, are $\sim 4 \times 10^{-4}$ G. Hence for large-angle scattering of particles with momentum 10^{16}–10^{19} eV/c (where c is the speed of light) we need structures separated by corresponding λ-values that are 1 pc–1 kpc. But large-scale inhomogeneities in synchrotron emission are seen[14] in the jet of 3C120, for example, on scales of ~ 10 mas–10 arcsec, corresponding approximately to these λ-values. Whether these structures are individual small-scale shocks or instabilities or other nonlinear wave effects, they must all feed equivalent-scale inhomogeneities into the hotspot shock at the jet termination.

Although observation of relativistic jet speeds is only convincing near the core and according to some calculations there is a significant slowing before the hotspot, the data are still consistent with maintenance of relativistic speeds throughout[14], and one theory involves re-acceleration of a plasma[15]. Observation of one-sided jet sources reveal more depolarization on the opposite (non-jet) side[16]. This depolarization is continuous in angular dependence and, in one case at least, arises from a foreground effect. Doppler beaming as a reason for one-sidedness and relativistic flow on all length scales are likely explanations[17]. Other parameters for the shock working surface are taken from Laing[18] who found field strengths of $\sim 4 \times 10^{-4}$ G at the hotspot and dimensions of this region of ~ 20 kpc from a survey of AGN jets. The field tended to be parallel to the jet, before and within the hot spot, suggesting a parallel-shock model.

For particles of rigidity 10^{19} V accelerated by a planar parallel shock, $V_1 = 0.96c$, $V_2 = 0.32c$, $\lambda = 41\rho_c$ with a 4×10^{-4} G mean field, the equation (2) acceleration time constant, enhanced by the calculated factor of 13.5 is $\approx 2 \times 10^4$ yr. This is short compared with the proton synchrotron and 2.7-K photoionization loss times of 9×10^7 yr and 10^{10} yr respectively. The diffusive loss time, T_d from the medium over a distance R of 10 kpc is 2×10^5 yr according to $T_d \approx 3R^2/4\lambda c$, which is still longer than the acceleration time At 10^{20} V, however, $\lambda = 10$ kpc according to our model, so diffusive acceleration is not likely at this rigidity Iron nuclei at rigidities $<10^{19}$ V, however, can supply the energy for the 10^{19}–10^{20}-eV particles

Following Ginzburg[19,20], the origin of 10^{17}–10^{20}-eV cosmic rays is due to acceleration within the local supercluster (~30 Mpc in size) in which conservatively ten AGNs may be described by the parameters adopted here An individual AGN power output of $2.6\times10^{51}/T$ erg s^{-1} is required to explain the observed $>10^{17}$-eV spectrum for a loss time T in the supercluster Taking an intercluster field of 10^{-8} G yields a diffusive loss time of 2×10^9 yr at 10^{17} eV, and nuclear collision and photoionization losses are negligible The required jet power into $>10^{17}$-eV particles of 5×10^{34} erg s^{-1} is small compared to that seen in the radiofrequency range ($\leq10^{46}$ erg s^{-1}) and even if the accelerated, low-energy cosmic-ray gas significantly loads the jet because of wave-particle interaction, the high end of the spectrum can still be treated by the test-particle approach

The actual spectrum of $>10^{17}$-eV cosmic-ray protons expected for our galaxy on the basis of the above model depends on the source spectrum, the momentum-dependent diffusion in the supercluster and the spectrum of source sizes For a single source at the centre of a homogeneous diffusing medium with a free-escape boundary at R and a catastrophic particle-loss time constant T_e, the propagation equation

$$\frac{\lambda c}{3}\nabla^2 n(p,r) - \frac{n}{T_e} = -Q\delta(r) \qquad (3)$$

has as a leading term in the $r \ll R$ solution, $n \propto \lambda^{-1}$ (ref 19) Here, Q is the source function, r is the distance from the centre of the diffusing medium and $\delta(r)$ is the Dirac function Thus the dominance of diffusive loss in the supercluster is expected to steepen the calculated differential-source spectrum, which has a p^{-1} dependence, by one power of p, because $\lambda \propto p$ If, in addition, the upper cutoff rigidity of each of the AGN hotspot contributions increases with available jet energy, there is a further spectral steepening The 10^{17}–10^{20}-eV cosmic-ray spectrum is believed to be $n \propto p^{-3}$, although a heavy-nuclei origin of the highest-energy air showers would require a further steepening Some contribution to the overall spectrum below the knee in the primary cosmic-ray spectrum is expected, but this is not important if the escape probability from the galaxy reduces to a $p^{-0.5}$ dependence and the energetic-particle loading of the accelerator eventually sets in We note that, experimentally, this feature may extend as low as 10^{13} eV ☐

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1 Begelman M C Blandford R D & Rees M J *Rev Mod Phys* **56**, 255–351 (1984)
2 Hillas A M *A Rev Astr Astrophys* **22**, 425–444 (1984)
3 Krimsky G F *Dokl Akad Nauk SSSR* **234**, 1306–1309 (1977)
4 Axford W I Leer E & Skadron G *Proc 15th Int. Conf on Cosmic Rays* Vol 11 (ed Christou C Ya) 132–137 (Bulgarian Acad Sci Sofia 1977)
5 Bell A R *Mon Not R astr Soc* **182**, 147–156 (1978) **182**, 443–455 (1978)
6 Blandford R D & Ostriker J P *Astrophys J* **211**, 793–808 (1977)
7 Peacock J A *Mon Not. R astr Soc* **196**, 135–152 (1981)
8 Bogdan T J & Webb G M *Mon Not. R astr Soc* **229**, 41–52 (1987)
9 Kirk J G & Schneider P *Astrophys J* **322**, 256–265 (1987)
10 Quenby J J *Space Sci Rev* **37**, 201–267 (1984)
11 Moussas X Quenby J J & Valdes Galicia J F *Solar Phys* **112**, 365–382 (1987)
12 Moussas X Quenby J J & Valdes Galicia J F *Astrophys Space Sci* **86**, 185–195 (1982)
13 Mavromichalaki H *et al* *Solar Phys* **116**, 377–390 (1988)
14 Walker R C Benson J M & Unwin S C *Astrophys J* **316**, 546–572 (1987)
15 Sol H Pelletier G & Asseo E *Mon Not. R astr Soc* **237**, 411–429 (1989)
16 Laing R A *Mon Not. R. astr Soc* **195**, 261–324 (1981)
17 Garrington S T Leahy J P Conway R G & Laing R A *Nature* **331**, 147–149 (1988)
18 Laing R A *Nature* **331**, 149–151 (1988)
19 Ginzburg V L *Proc 20th Int Conf on Cosmic Rays* Vol 7 (eds Kozyarivsky V A *et al*) 7–54 (Nauka Moscow 1987)
20 Ginzburg V L & Syrovatskii S *The Origin of Cosmic Rays* (Pergamon Oxford 1964)
21 Kirk J G & Schneider P *Astr Astrophys* **201**, 177–188 (1988)

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Does mass accretion lead to field decay in neutron stars?

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WHETHER or not neutron-star magnetic fields decay is a matter of current debate The recent observation[1,2] of cyclotron lines from γ-ray-burst sources, thought to be relatively old neutron stars, indicates that they are strongly magnetized and therefore that their fields have not decayed One interpretation of the correlation observed[3] between the strength of the magnetic field and the mass accreted by the neutron star is that mass accretion may itself lead to the decay of the magnetic field. Adopting the hypothesis of accretion-induced field decay, we calculate here the consequent evolution of a neutron star's spin and magnetic field The results are consistent with observations of binary and millisecond radio pulsars. Thermomagnetic effects[4] could provide a possible physical mechanism for such accretion-induced field decay.

Statistical analyses[5,6] of ~400 radio pulsars and the study[3] of the origin and evolution of magnetized neutron stars in binary systems are consistent with the assumption that neutron stars are born with magnetic fields of 10^{12} G which then decay with a time constant of $5-10\times10^6$ yr Estimates of age and magnetic field strength of binary millisecond radio pulsars, however, require[7] that the field decay, if it occurs, should stop or proceed much more slowly on timescales of $\geq10^9$ yr, at field strengths $\leq10^{10}$ G Pulsar models in which magnetic fields do not decay but align with the rotation axis are shown to also be consistent with the observed properties of radio pulsars[8-10]

Ohmic dissipation of electrical currents in the crust has been thought to be the physical cause for field decay[11] Recent calculations[12] of the ohmic decay of dipolar magnetic fields, however, have shown that the field does not decay exponentially as has been assumed in most statistical analyses of radio pulsars and that, if the field occupies the entire crust, it decays by less than a factor of 100 in a Hubble time

If the strong magnetic fields of neutron stars do not decay, then the origin of the weak magnetic fields of less than 10^{10} G, found in binary and millisecond radio pulsars[13], remains to be explained Neutron stars in binary and millisecond radio pulsars are thought to be formed by the iron-core collapse of massive stars or by the accretion-induced collapse of massive white dwarfs (for reviews see refs 14, 15) In one model for the weak field and rapid rotation of millisecond radio pulsars it is argued that it is simply white dwarfs[16-18] or iron cores of massive stars[19] having the appropriate field strength and angular momentum that give birth to the observed millisecond radio pulsars after a collapse In another model, mass accretion in low-mass X-ray binaries, which are assumed to be progenitors of millisecond radio pulsars, is proposed to cause both the field decay[20] and the spin-up[21] In fact, Taam and van den Heuvel[3] found a possible inverse correlation between the magnetic field strength and the estimated total mass of accreted matter for binary X-ray sources and for binary and millisecond radio pulsars This inverse correlation supports the latter model A study of the evolution of magnetic fields in the crust of a neutron star[4] showed further that the inward heat flux caused by mass accretion gives rise to thermomagnetic effects that could remove the strong magnetic field of a neutron star Here we examine further the possibility that magnetic fields of neutron stars decay only when neutron stars undergo mass accretion by considering γ-ray bursts and the periods and field strengths of binary and millisecond radio pulsars

The recent discovery[1 2] of cyclotron absorption lines with 20-keV and 40-keV energies in γ-ray bursts revealed that the central objects of γ-ray bursters are, as indicated in some theoretical works[22,23], strongly magnetized neutron stars with magnetic fields of 10^{12} G The statistical arguments indicate $\sim 10^7$ yr (refs 24, 25) or $\gg 10^7$ yr (ref 26) for the age of neutron stars in γ-ray bursters depending on the assumed distance When combined with the high field strength, the latter age estimate contradicts the field-decay hypothesis whereas the former does not

We note that the absorption lines were seen only in a limited portion of the burst[1] This can also be used to test the field-decay hypothesis because the presence of the cyclotron lines may depend sensitively on the configuration of the magnetic field relative to the line of sight[27] If this is the case, the 5-10-s duration of the cyclotron-absorption feature indicates the rotation period of $\geq$10-20 s This rotation period and the field strength put the γ-ray burst sources into the category of the 'turned-off' pulsars[6] If the spin-down of the neutron star is caused by the magnetic field, which decays exponentially on a timescale of τ_B, the variation of the rotation period P with time t is represented by $P^2 = AB_0^2\tau_B[1-\exp(-2t/\tau_B)]+P_0^2$ where B_0 and P_0 are initial field strength and rotation period, respectively, and the constant A is $9\ 8\times 10^{-40}$ s G^{-2} for a stellar moment of inertia of 10^{45} g cm^2 and radius of 10^6 cm To attain the rotation period of $\geq$10-20 s from the initial value of $\leq$1 s, magnetic braking requires the initial field strength of the neutron star to be larger than $\sim 2\times 10^{13}$ G if $\tau_B \sim 10^7$ yr This value of the initial field seems large, compared to the average values of $\sim 10^{12}$ G indicated by the statistical analyses of radio pulsars Therefore we pursue here the other possibility—that the magnetic fields of γ-ray burst sources, which might be isolated neutron stars, do not decay

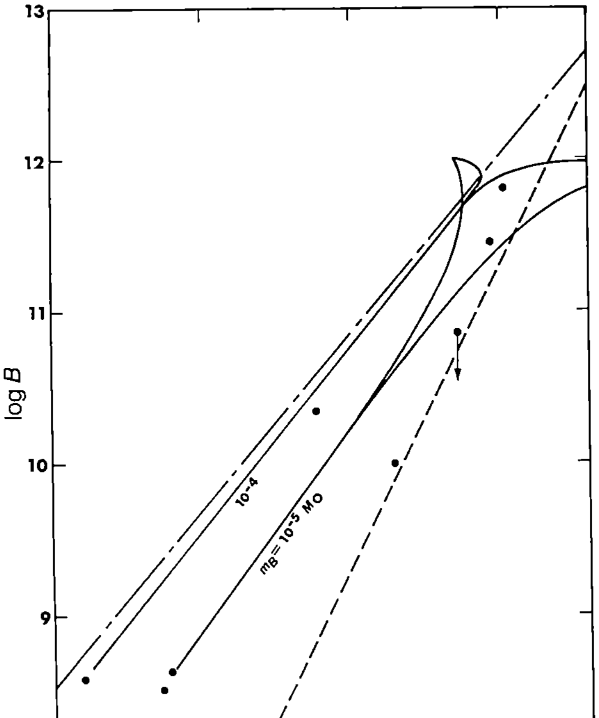

FIG 1 Evolutionary tracks (solid lines) of neutron stars in the plot of magnetic field as a function of rotation period Rotation periods and magnetic fields become smaller with accreted mass and hence with increasing time After mass accretion stops, the parameters develop horizontally to the right The initial magnetic field is taken as $B_0=10^{12}$ G, the initial rotation period is chosen as $P_0=0\ 5$ or 100 s, and the mass accretion rate is fixed at $M=1\ 1\times 10^{18}$ g s^{-1} The mass, radius and moment of inertia of the neutron star are taken as 1 4 $M_\odot$, 106 cm, and 10^{45} g cm^2, respectively The dash-dotted and dashed lines denote the equilibrium rotation[28] and pulsar-death lines[6], respectively The filled circles represent the positions of binary and millisecond radio pulsars in this diagram

As already mentioned, the weak magnetic fields of $\leq 10^{10}$ G are found in binary and millisecond radio pulsars[13], these magnetic fields are plotted against rotation periods in Fig 1 In the resurrected-pulsar model[21] the progenitors of millisecond radio pulsars are the low-mass X-ray binaries and their rapid rotations are the consequence of the spin-up that is due to the mass accretion in binary systems We consider the weak magnetic field and the rapid rotation of these radio pulsars in terms of the hypothesis that mass accretion leads to both spin-up and field decay We assume that the magnetic field decays with accretion as

$$B=\frac{B_0}{1+\Delta M/m_B}=\frac{B_0}{1+Mt/m_B} \qquad (1)$$

where B is the field strength at time t, ΔM the accreted mass, m_B the mass constant for the field decay and M the accretion rate Equation (1) fits well the inverse correlation between the magnetic field strength and the estimated mass of the total accreted matter for binary X-ray sources and binary and millisecond radio pulsars[3] As already noted[3], this inverse correlation is also consistent with the simple field-decay hypothesis if the larger amount of accreted matter is interpreted simply to reflect the greater age of the neutron star Although we do not advocate any physical model leading to equation (1), we do assume that a direct relation between field loss and accreted mass exists We note that equation (1) represents a change in the whole field, not just the component perpendicular to the rotation axis Using the formula given by Ghosh and Lamb[28] for the accretion torque, the variation of rotation period is described by $P = -0\ 11I^{-1}(GM)^{3/7}(BR^3)^{2/7}nM^{6/7}P^2$ where n is the dimensionless accretion torque (see ref 28), R the radius, I the moment of inertia and G the gravitational constant The calculated evolutionary tracks in the plot of the magnetic field against rotation period are shown by the solid lines in Fig 1 The rotation periods and magnetic fields decrease with time We ended our calculations when the inner edge of the accretion disk reached the surface of the neutron star When the mass constant for the field decay is $m_B \geq 10^{-3}M_\odot$, the evolution proceeds along the equilibrium rotation line[28] (dash-dotted line in Fig 1), where the spin-up torque that is due to accreting matter and the spin-down torque that is due to magnetic field are balanced This is because the timescale of the field decay is longer than the spin-up timescale After mass accretion stops, the parameters develop horizontally towards the right in Fig 1 If $m_B \geq 10^{-4} M_\odot$, the calculated evolutionary tracks are consistent with B and P of binary and millisecond radio pulsars These evolutionary tracks are also approximately consistent with the B and P of low-mass binary X-ray sources such as suggested from the beat-frequency model[29] for the quasiperiodic X-ray oscillations (see ref 30 for a review) Note that the possible inverse correlation[3] between B and ΔM is represented well by equation (1) with $m_B \sim 10^{-4} M_\odot$ Hence, if we adopt $m_B \sim 10^{-4} M_\odot$, the accretion-induced field-decay hypothesis represented by equation (1) is consistent with B, P and ΔM as observed or estimated for binary and millisecond radio pulsars and binary X-ray sources

The magnetic fields of single radio pulsars are in the range of 10^{11}-10^{13} G with a distribution peak around 10^{12} G The accreted mass of interstellar matter onto a single radio pulsar is negligible compared to $m_B \sim 10^{-4} M_\odot$ Hence, according to the accretion-induced field-decay hypothesis, the present field strengths of single radio pulsars should be equal to their initial values But this assertion conflicts with the results of the statistical analyses[5 6] of radio pulsars, which suggest that surface fields decay on a timescale of $5-10\times10^6$ yr Pulsar statistics depend, however, on the assumptions made for the radio luminosity law, the braking index, the magnetic-field evolution, the distribution of initial field strength and so on To resolve the above conflict, the statistical analysis under the assumption of no field decay should be conducted, examining especially the dependence on the assumptions for the above properties

Mass accretion of neutron stars is likely to give rise to the inward heat flow through the crust The thermomagnetic effects in the crust that arise from this inward heat flux, such a suggested in ref 4, have been invoked as a possible mechanis of the accretion-induced field decay The formula of ref predicts, in its simplest form, a stronger dependence of the fiel decay on time than does equation (1) The field-decay formul based on thermomagnetic effects, however, may depend on th structure of the crust, the accretion process and the histor of the heat flux A more detailed study of the thermomagnetic effects in the crust is needed to resolve the controversia issue of the field decay in neutron stars Other possible mechanisms for the accretion-induced field decay should also b explored □

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1 Murakami T *et al* *Nature* **335,** 234-235 (1988)
2 Fenimore E E *et al* *Astrophys J* **335,** L71-L74 (1988)
3 Taam R E & van den Heuvel E P J *Astrophys J* **305,** 235-245 (1986)
4 Blondin J M & Freese K *Nature* **323,** 786-788 (1986)
5 Lyne A G Manchester R N & Taylor J H *Mon Not R astr Soc* **213,** 613-639 (1985)
6 Stollman G M *Astr Astrophys* **178,** 143-152 (1987)
7 Kulkarni S R *Astrophys J* **306,** L85-L89 (1986)
8 Candy B N & Blair D G *Astrophys J* **307,** 535-539 (1986)
9 Cheng A F *Astrophys J* **337,** 803-813 (1989)
10 Kundt W *Comments Astrophys* **12,** 113-121 (1988)
11 Ostriker J P & Gunn J E *Astrophys J* **157,** 1395-1417 (1969)
12 Sang Y & Chanmugan G *Astrophys J* **323,** L61-L64 (1987)
13 Kulkarni S R in *Physics of Neutron Stars and Black Holes* (ed Tanaka Y) 37-53 (Universal Academy 1988)
14 Van den Heuvel E P J in *The Origin and Evolution Neutron Stars* (eds Helfand D J & Huang J H) 393-406 (Reidel Dordrecht 1987)
15 Nomoto K in *The Origin and Evolution Neutron Stars* (eds Helfand D J & Huang J-H) 282-303 (Reidel Dordrecht 1987)
16 Michel F C *Nature* **329,** 310-312 (1987)
17 Chanmugan G & Brecher K *Nature* **329,** 696-698 (1987)
18 Grindlay J E & Bailyn C D *Nature* **336,** 48-50 (1988)
19 Brecher K & Chanmugan G *Nature* **338,** 712 (1989)
20 Van den Heuvel E P J van Paradijs J A & Taam R E *Nature* **322,** 153-155 (1986)
21 Alpar M A Cheng A F Ruderman M A & Shaham J *Nature* **300,** 728-730 (1982)
22 Michel F C *Astrophys J* **290,** 721-727 (1985)
23 Liang E *Comments Astrophys* **12,** 35-49 (1987)
24 Van Paradijs J A *Mon Not R astr Soc* **238,** 45-48 (1989)
25 Hartmann D Epstein R I & Woosley S E *Astrophys J* (in the press)
26 Wang J C L *et al* *Phys Rev Lett.* (submitted)
27 Bussard R W & Lamb F K in *Gamma Ray Transients and Related Astrophysical Phenomen* (eds Lingenfelter R E Hudson H S & Worrall D M) 189-200 (American Institute of Physic 1982)
28 Ghosh P & Lamb F K *Astrophys J* **234,** 296-316 (1979)
29 Alpar M A & Shaham J *Nature* **316,** 239-241 (1985)
30 Lewin W H G van Paradijs J & van der Klis M *Space Sci Rev* **46,** 273-377 (1988)

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A hierarchy of catastrophes as a succession of stability limits for the crystalline state

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MANY theories of melting have focused on some form of instability or catastrophe delimiting the range of stability of the crystalline state These are exemplified by the vibrational instability proposed by Lindemann[1] and the elastic shear instability of Born[2]. Fecht and Johnson[3] suggested that an entropy catastrophe delimits the stability range of both a superheated crystal and a supercooled liquid Drawing on Kauzmann's idea[4] that the glass transition pre-empts a catastrophe point at which the entropy of supercooled liquid equals that of the crystal, they suggest that a second catastrophe point occurs when the entropy of the superheated crystal equals that of the liquid phase, and that this is pre-empted by melting Here I argue that these entropy catastrophes are an outer bound on the stability limits of the crystalline and liquid phases, and that a hierarchy of inner catastrophes occurs at reduced superheatings or supercoolings

Kauzmann showed that, with progressive supercooling, the entropy of a liquid tends towards the entropy of the crystalline state Were they to cross at some temperature T_g, one would be faced with Kauzmann's paradox that, at temperatures below T_g, the entropy of the ordered crystal would exceed that of the disordered liquid This situation is prevented by the occurrence of the glass transition Although the glass transition may be loosely termed a 'kinetic transition', reflecting the fact that the structural relaxation time becomes so long that fluidity is lost, it is seen from Kauzmann's ideas that there is an underlying instability The kinetic character means that the observed transition point depends on the cooling rate but, in the limit o infinitely slow cooling, the transition point must remain greate than or equal to the instability point Moreover, one consequence of the free-volume theory of the glass transition is the prediction of an underlying first- or second-order transition[5] Thus, there appears a succession of catastrophe barriers to supercooling, first kinetic (corresponding to the glass transition), then thermodynamic and finally entropic (corresponding to the Kauzmann point) If one barrier were to be surmounted, the next would provide the natural ultimate limit for further supercooling Here I show that further barriers may be deduced and that a similar hierarchy of catastrophes occurs in relation to superheating of

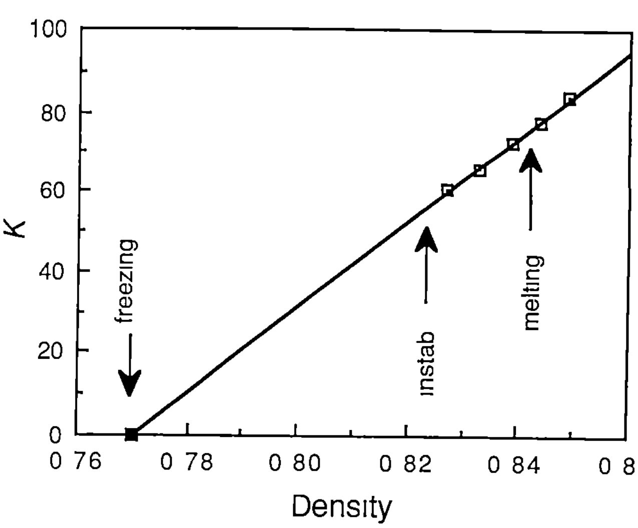

FIG 1 The Kosterlitz-Thouless parameter K as a function of reduced density for a computer-simulated two-dimensional crystal of Lennard-Jones atoms The solid data point shows that the crystalline data extrapolate to zero at the freezing density Densities are quoted in reduced units The equilibrium freezing and melting points are indicated together with the shear-instability point for maximum superheating

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Permanent address Physics and Engineering Laboratory DSIR PO Box 31313 Lower Hutt New Zealand crystals, that is, to melting and the metastable extent of the crystalline phase

The interpretation of melting in terms of a one-phase instability model carries with it a number of problems Born's idea[2] that the shear modulus falls with increasing temperature becoming zero at the melting point T_m is evidently incorrect, or at least incomplete, as crystals still possess rigidity close to T_m Mechanical instability models imply second-order melting with marked pre-melting behaviour[6] rather than the observed first-order melting with little or no pre-transitional behaviour[7] Finally, the notion of crystalline instability refers only to the properties of the crystalline phase and any rigorous treatment of melting must also refer to the properties of the liquid phase The melting point is defined as the temperature and pressure at which the Gibbs free energy of the two phases are equal and beyond which thermodynamic instability occurs Nonetheless the notions of thermodynamic instability and mechanical instability can be harmonized and the two shown to coincide in a special sense To develop these ideas I use two previously reported results

First, if one examines the shear or rigidity moduli as functions of molar volume for a large range of cubic or close-packed solids, one of the moduli extrapolates to zero at the volume of the liquid at the freezing point[8 9] To illustrate this I have examined computer-simulation data for a two-dimensional Lennard-Jones system and find exactly the same result as previously reported[8 9] for real three-dimensional systems The data are the results of Monte Carlo calculations[10] of the elastic Lamé coefficients μ and λ for a 529-atom system close to the triple-point The Kosterlitz–Thouless parameter K defined by

$$K=\frac{4a^2}{kT_\mathrm{m}}\frac{\mu(\mu+\lambda)}{(2\mu+\lambda)} \tag{1}$$

is plotted in Fig 1 as a function of density Here a is the lattice parameter and k is Boltzmann's constant In Fig 1 the equilibrium thermodynamic melting density and freezing density are marked, together with the instability density at which the superheated crystal spontaneously melts by the generation of dislocations This instability point corresponds to a value of K close to the value 16π predicted theoretically[11] The value of K for the crystal extrapolates to zero at the freezing point density This means that Born's idea, that melting coincides with the vanishing of a shear modulus, is essentially correct except that this occurs near the freezing point rather than the melting point (note that these do not coincide in T, P, V phase space) We have argued[8] that the appearance of the communal entropy, once rigidity is lost, allows the system to discontinuously transform from a higher density to this critical density at the freezing point where the rigidity modulus vanishes The shear instability, properly interpreted, thus naturally gives rise to a first-order melting transition for two dimensions as well as for three dimensions In conventional isothermal melting the instability occurs within the solid–liquid interface, a region that is effectively superheated as its density is lower than that of the crystal

Second, we have shown that the entropy change ΔS on isochoric (that is constant volume) melting for a large range of simple crystalline materials is a constant equal to $\sigma R \ln 2$ where σ takes the value 1 for monatomic systems, 2 for binary systems and so on[12 13] This has been demonstrated for the inert gases, alkali metals, alkali and alkali earth halides as well as a variety of computer-simulated systems that is, for all systems with approximately spherical atoms Subsequent investigations of molecular-dynamics-simulated systems showed that this entropy change was just the communal entropy of the dense liquid[13] Thus, to a good approximation, the only difference in entropy between liquid and crystal at the same density is the communal entropy of the liquid This reduces to zero in at least three distinct transitions[13,14] the glass transition[14] (and as a consequence there is essentially no entropy difference between glass and crystal at the same density), in the superfluid transition[13] for ^{4}He, and in the fast-ion transition of an ionic crystal[13]

Where liquid and crystal are not at the same density the entropy difference may be well approximated by noting that the isothermal volume dependence of the entropy is given by $(\partial S/\partial V)_T=\alpha_\mathrm{v}\beta_T$ where α_v is the volume coefficient of thermal expansion and β_T is the isothermal bulk modulus The product $\alpha_\mathrm{v}\beta_T$ is nearly constant from above the Debye temperature to the melting point and takes nearly the same value in the melt It is not unexpected, therefore, that we find

$$\Delta S_\mathrm{m}=\alpha_\mathrm{v}\beta_T\Delta V_\mathrm{m}+\sigma R\ln 2 \tag{2}$$

an excellent approximation for the entropy change on melting This relation is well satisfied for all of the simple systems noted above with approximately spherical atoms[12]

Some simple conclusions using the above results Figure 2 is a reproduction of Fecht and Johnston's[3] diagram of the temperature dependence of the entropy of crystalline (curve C–C′) and liquid (curve L–L′) aluminium (Their calculated extrapolations are estimates only and aluminium, because of its non-spherical atoms, is not a good choice for applying the above simple phenomenology The diagram is, however, appropriate for illustrative purposes) Fecht and Johnston identified two catastrophe points, marked T_g^s and T_m^s in the figure, at which the entropies of the two phases are equal By subtracting the communal entropy, 0 69 R, from the entropy in the liquid phase I obtain the light solid line annotated G–G′ This represents the entropy of the liquid over the entire temperature range if the liquid were non-diffusive, that is, if the atoms were constrained to remain within their individual shells of neighbouring atoms Such a system is equivalent to a glass The entropy on this curve, according to equation (2), differs from the crystalline entropy by a volume-dependent term only, and consequently at the two

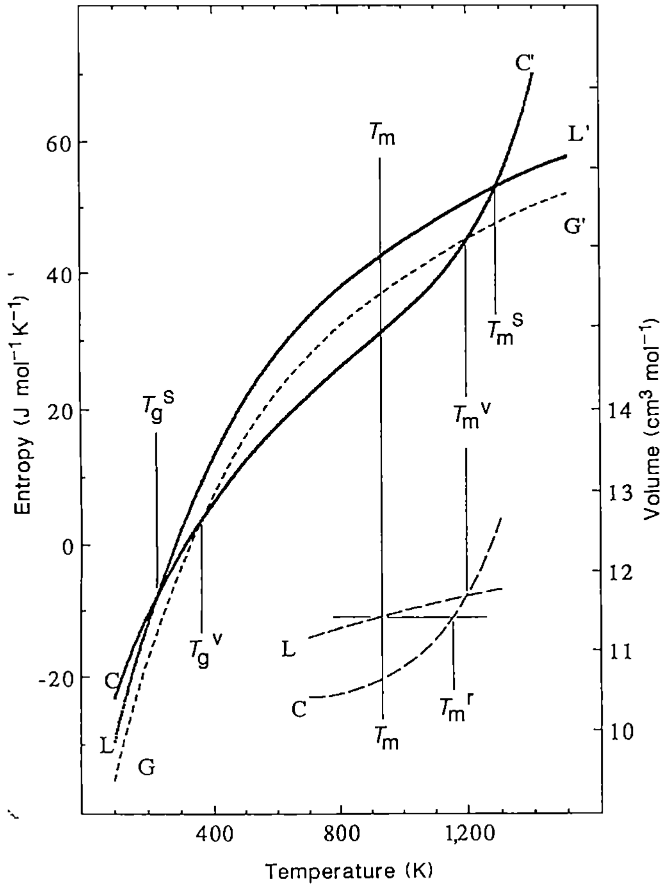

FIG 2 The calculated entropy and molar volume for aluminium in the crystalline (C) and liquid (L) phases at 1 bar See text for explanation

points marked T^{v}_{g} and T^{v}_{m}, where the curve G-G′ intersects C-C′, the crystalline and liquid phases have the same molar volume or density Beyond these points the crystal would be less dense than its liquid phase at the same temperature and for a close-packed crystal this is quite unexpected These two points therefore represent further catastrophe points, the boundaries of regions of paradox comparable to those of Kauzmann (There are materials for which the density of the liquid exceeds that of the crystalline solid[6], including Ga, Bi, Sb, H_2O and KI at elevated pressure, but these cases arise from the occurrence of a very open crystalline structure)

Having identified the two isochoric catastrophe points at which crystal and liquid possess the same density and which preempt the isentropic catastrophe points, I can estimate from these entropy curves the molar volumes of the two phases as they vary with temperature The object of this last calculation is to locate the point of rigidity instability which, according to the above ideas, occurs where the density of the superheated crystal equals that of the liquid at the freezing point For a non-isothermal process

$$\begin{aligned}\Delta S &= \alpha_v\beta_T\Delta V + \int (C_v/T)\,\mathrm{d}T \\ &\approx \alpha_v\beta_T\Delta V + C_v\ln(T/T_0)\end{aligned}\tag{3}$$

The latter approximation applies if C_v is constant, as may be assumed well above the Debye temperature Taking T_0 as T_m, the crystalline and liquid volumes are estimated from curves G-G′ and C-C′ by subtracting $C_v\ln(T/T_m)$ and dividing by $\alpha_v\beta_T$ These are plotted as the dashed lines in Fig 2, to which the volume scale on the right-hand side applies The point T at which the volume of the superheated crystal equals the volum of the liquid at the freezing point is marked This is the poi at which the rigidity modulus vanishes and shear instabili occurs, and this precedes both other catastrophe points T^{v}_{m} an T^{s}_{m} I note that the calculations of Boyer[15] indicate that th occurrence of a shear instability drives a preemptive instabilit in the compressibility Thus, for crystalline superheating a su cession of catastrophes occur in the sequence thermodynami (equilibrium melting), elastic compressibility, elastic rigidit isochoric (equal volumes for crystal and liquid) and finall entropic points I believe that the last two catastrophes are n observable as they are preceded by the rigidity catastrophe whic is the underlying cause of melting [

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1 Lindemann F A *Z Phys* **11**, 609–615 (1910)
2 Born M *J Chem Phys* **7**, 591–601 (1939)
3 Fecht H J & Johnson W L *Nature* **334**, 50–51 (1988)
4 Kauzmann W *Chem Rev* **43**, 219–256 (1948)
5 Cohen M H & Grest G S *Phys Rev* B**20**, 1077–1098 (1979)
6 Ubbelohde A R *The Molten State of Matter* (Wiley Chichester 1978)
7 Tallon J L Robinson W H & Smedley S I *J phys Chem* **82**, 1277–87 (1978)
8 Tallon J L Robinson W H & Smedley S I *Nature* **266**, 337–338 (1977)
9 Tallon J L *Phil Mag* A**39**, 151–161 (1979)
10 Abraham F F *Phys Rep* **80**, 339–374 (1981)
11 Kosterlitz J M & Thouless D J *J Phys* **C5**, L124–126 (1972) **C6**, 1181–1191 (1973)
12 Tallon J L & Robinson W H *Phys Lett* A**87**, 365–368 (1982)
13 Tallon J L *Phys Rev* B**29**, 4153–4155 (1984)
14 Madsen J U & Cotterill R M J *Phys Lett* A**83**, 219–220 (1981)
15 Boyer L L *Phase Transitions* **5**, 1–48 (1985)

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Interhemispheric asymmetry in climate response to a gradual increase of atmospheric CO_2

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THE transient response of a coupled ocean–atmosphere model to an increase of atmospheric carbon dioxide has been the subject of several studies[1–8]. The models used in these studies explicitly incorporate the effect of heat transport by ocean currents and are different from the model used by Hansen *et al*[9] Here we evaluate the climatic influence of increasing atmospheric carbon dioxide using a coupled model recently developed at the NOAA Geophysical Fluid Dynamics Laboratory. The model response exhibits a marked and unexpected interhemispheric asymmetry In the circumpolar ocean of the Southern Hemisphere, a region of deep vertical mixing, the increase of surface air temperature is very slow. In the Northern Hemisphere of the model, the warming of surface air is faster and increases with latitude, with the exception of the northern North Atlantic, where it is relatively slow because of the weakening of the thermohaline circulation

The model we use here consists of general circulation models (GCMs) of the atmosphere and oceans and a simple model of the continental surface that involves the budgets of heat and water It is a global model and includes realistic geography The atmospheric component of the model used here is very similar to the model used in ref 10 The seasonally varying insolation at the top of the model atmosphere is taken into account in calculating the solar radiation Whenever the relative humidity at a grid point exceeds a critical value (99%), the grid box is filled with cloud, otherwise, it is free of cloud The atmospheric GCM uses a spectral transform method in which the horizontal distributions of predicted variables are represented by spherical harmonics (15 associated Legendre functions for each of 1 Fourier components) and grid-point values For vertical-finite difference calculations, nine unequally spaced levels are chosen The ocean GCM uses a full finite-difference technique and ha a regular grid system with 4 5°×3 75° (latitude×longitude spacing and 12 vertical levels[11] The atmospheric and oceani components of the model interact with each other continuousl through the exchange of heat, water and momentum fluxes

Two 100-year integrations of the coupled model were performed In the first integration the atmospheric concentratio of carbon dioxide increased by the rate of 1% yr^{-1} (compounded) The CO_2 concentration remains unchanged in the secon integration (the control) We have evaluated the influence o

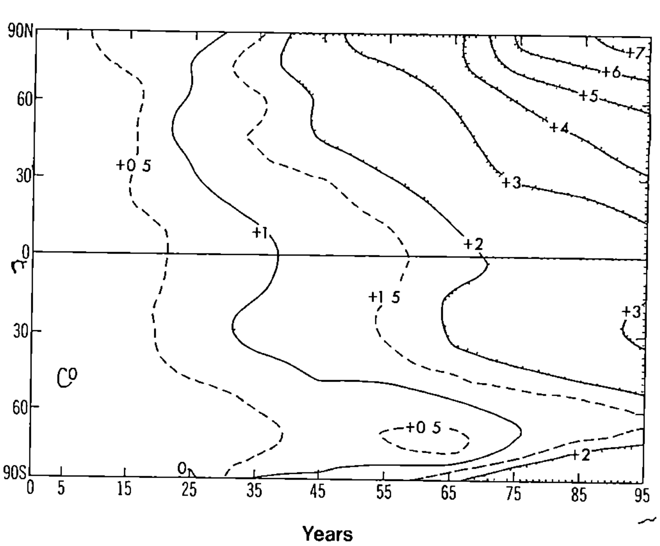


FIG 1 Latitude–time distribution of the difference in zonally averaged, decadal-mean surface air temperature (°C) between the two integrations Zonal-mean surface air temperature is averaged over the decades centred at the fifth, fifteenth , and ninety-fifth year of the experiment

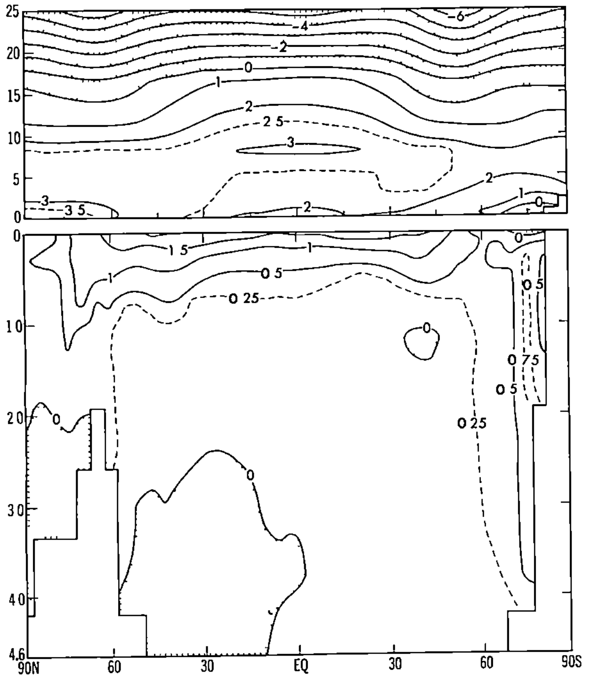


FIG. 2 Zonal-mean difference in temperature (°C) of the ocean–atmosphere model between the two integrations. The difference represents the decadal average over years 61–70. The atmospheric data have been interpolated to isobaric surfaces.

gradually increasing atmospheric carbon dioxide upon the coupled system from the difference between the first and second (control) integrations. If the CO_2 increase of 1% yr^{-1} begins in AD 1958, the doubling of CO_2 concentration would be achieved around AD 2030. Such an increase of CO_2 is larger than its projected increase but is approximately equal to the present rate of increase of the combined thermal forcing of all greenhouse gases (other than water vapour)[12].

The initial conditions for both integrations have realistic seasonal and geographical distributions of surface temperature, surface salinity and sea ice, with which both the atmospheric and oceanic components of the model are nearly in equilibrium. This quasi-equilibrium condition was obtained by separate time integrations of these two components of the model using observed surface conditions. The convergence of the oceanic component towards equilibrium was accelerated by the method described in ref. 13.

To avoid the systematic climate drift caused by the bias of the model during the two 100-year integrations, the oceanic surface fluxes of heat and water are adjusted seasonally and geographically, but not interannually, using the method described in ref. 14. Identical adjustments are applied to both the first and the second integrations defined above. By contrast to recent studies[4,8], no systematic trends are evident during the control integration, indicating that the initial condition is close to stable equilibrium and the flux adjustments are working as expected.

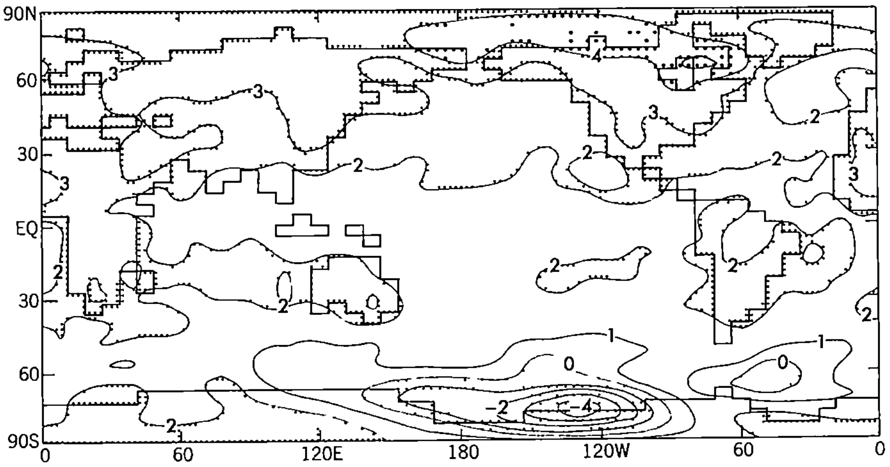
Figure 1 shows the latitude–time distribution of the CO_2-induced change of zonally averaged, decadal-mean surface air temperature during the 100-year experiment. In the Northern Hemisphere the warming of surface air increases with latitude, partly because of the poleward retreat of both snow cover and sea ice which have high surface albedo. The relatively large warming in high northern latitudes is essentially confined to the lower troposphere because of the stable stratification. This is indicated in Fig. 2 which shows the difference in zonal-mean temperature between the two integrations for the seventh decade of the experiment. The ocean warming is essentially confined to the upper layer, except near Antarctica. It also increases with latitude in the Northern Hemisphere, but becomes small near the North Pole which is covered by sea ice.

The meridional profile of the near-surface warming in the Northern Hemisphere is qualitatively similar to the difference between two equilibrium climates of a model with the normal and the above-normal concentrations of atmospheric CO_2 (See, for example, refs 15, 16, studies that were conducted using an atmosphere/mixed-layer-ocean model and atmosphere–ocean GCM, respectively.)

Over the northern North Atlantic, the increase of surface air temperature is significantly smaller than the zonal average. This is evident in Fig. 3 which shows the geographical distribution of the difference in decadal-mean surface air temperature between the two integrations for the seventh decade of the experiment. Because of the increase in runoff and precipitation over evaporation, surface salinity decreases and the stability of upper ocean layer increases in the northern North Atlantic. Thus, the thermohaline circulation of the North Atlantic is weakened by ~25% by the seventh decade of the experiment. The weakening is shown in Fig. 4 which illustrates the meridional circulation zonally averaged over the entire oceanic segment of a latitude circle. (Note the reduction from 16 to 12 Sv in the Northern Hemisphere.) Because of the weakening of the thermohaline circulation, the northward advection of warm and saline subtropical surface water is reduced, thereby reducing surface salinity further and making the increase of sea surface temperature relatively small over the northern North Atlantic Ocean as indicated in Fig. 3. The reduction of surface salinity stabilizes the near-surface layer and provides a feedback that further weakens the thermohaline circulation in the northern North Atlantic.

In an earlier study, Bryan and Spelman[3] investigated the

FIG. 3 The geographical distribution of the difference in surface air temperature (°C) between the two integrations averaged over years 61–70.



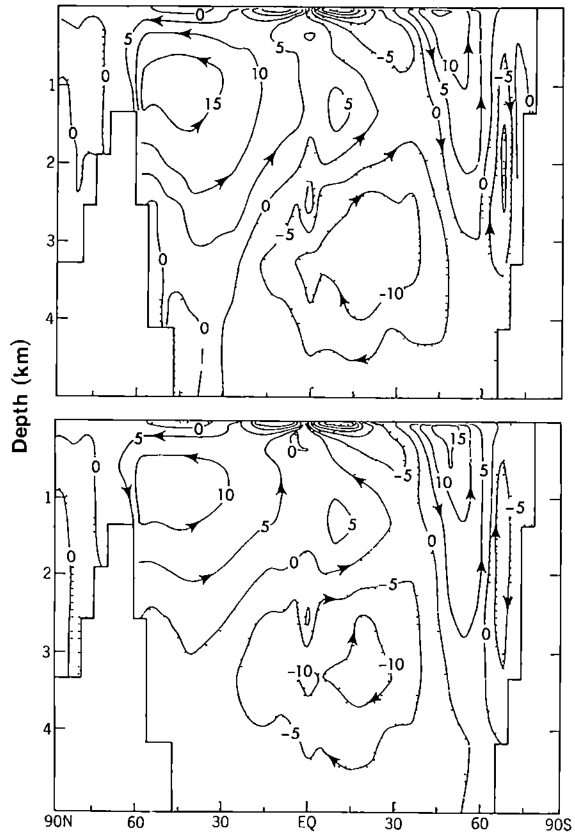


FIG 4 Streamlines of zonal-mean meridional oceanic circulation (in Sverdrups) averaged over the seventh decade (that is, years 61-70) of the experiment Top, the control integration with constant CO_2, bottom, the integration in which atmospheric CO_2 concentration is increased with the rate of 1% yr^{-1}

influence of an abrupt quadrupling of atmospheric CO_2 on the climate of an atmosphere-ocean model with a sector computational domain bounded by the Equator and two meridional boundaries They found that the thermohaline circulation of the model collapsed completely towards the end of the third decade of the experiment Washington and Meehl[8] noted that the response of their global model to a linear increase of atmospheric CO_2 was a weakening of the thermohaline circulation in the Atlantic Manabe and Stouffer[14] found that their global model has two stable equilibria, one with and one without the thermohaline circulation in the Atlantic Ocean Although we do not observe a complete collapse of the thermohaline circulation here, it is a possibility that deserves[17] further assesment

In the Southern Hemisphere, the CO_2-induced warming of surface air decreases with increasing latitude in sharp contrast to the situation in the Northern Hemisphere and to the results of equilibrium experiments The rate of the warming is particularly slow in the Antarctic circumpolar ocean A similar feature is noted and analysed in studies[6,7] that explored the effect of an abrupt doubling of the atmospheric CO_2 on coupled models with 'sector' and global computational domain, respectively The latter model is very similar to the present model except that it has annual-mean insolation The fraction of the area covered by oceans in the Southern Hemisphere is much larger than the corresponding fraction in the Northern Hemisphere Thus the effective thermal inertia of the Southern Hemisphere tends to be larger than in the Northern Hemisphere[18], accounting for the slower rise of surface air temperature in the former

Based upon a detailed study of the heat budget[6,7] there is another important mechanism responsible for the interhemispheric difference of effective thermal inertia particularly in high latitudes From 45°-65° S, the strong surface westerlies induce an Ekman drift current towards the Equator Because of the absence of a continental barrier in the upper oceanic layer, the zonal pressure gradient is small at the latitudes of the Drake Passage and the southward geostrophic return flow is weak beneath the surface mixed layer Instead, the surface drift currents are compensated by a deep downwelling north of the Drake Passage gap, southward geostrophic flow at the base of the gap, and deep upwelling south of the circumpolar current, as indicated in Fig 4 The existence of such an upwelling region is inferred[19,20] from water mass analysis A deep wind-driven cell is also simulated by eddy-resolving, high-resolution models[21] In addition to the wind-driven deep cell of meridional circulation described above, we note that (Fig 4) there is another cell circulating in the reverse direction in the immediate vicinity of Antarctica where deep convective overturning prevails, forming the Antarctic bottom water These deep cells of meridional circulation together with associated convective overturning and other sub-grid-scale mixing processes enhance the vertical mixing of heat over a thick ocean layer, thereby increasing the effective thermal inertia of the coupled ocean-atmosphere system This large effective thermal inertia is responsible for the very slow rise of the near-surface temperature in the circumpolar ocean of the model It is likely that a similar effective vertical mixing is also realized in the actual circumpolar ocean which is characterized by relatively small vertical variations of density and other trace substances such as phosphate and ^{14}C

The marked interhemispheric asymmetry of our result differs from the study of Schlesinger *et al*[4] in which an almost symmetric response was obtained A recent result[8] indicates smaller warming in the Southern than in the Northern Hemisphere However, the asymmetry is much less pronounced than in the present results This discrepancy may result from the fact that the wind-driven cell in the circumpolar ocean obtained in the earlier studies is much weaker and shallower than the cell simulated by the present model Obviously, a careful comparison of results is needed to determine the underlying cause of this discrepancy

Further evaluation of the mechanisms involved in the effect of ocean circulation on the CO_2-induced climate change is in progress The general applicability of the present results will be evaluated by conducting further simulations with different rates of CO_2 change and with models of higher computational resolution □

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1 Bryan K Komro F G Manabe S & Spelman M J *Science* **215**, 56-58 (1982)
2 Spelman M J & Manabe S *J geophys Res* **89**, 571-586 (1984)
3 Bryan K & Spelman M J *J geophys Res* **90**, 11679-11688 (1985)
4 Schlesinger M E & Jiang X *Climate Dynamics* **3**, 1-17 (1988)
5 Schlesinger M E Gates W L & Han Y J in *Coupled Ocean-Atmosphere Models* (ed Nihoul I C J) 447-478 (Elsevier Amsterdam 1985)
6 Bryan K Manabe S & Spelman M J *J phys Oceanogr* **18**, 851-867 (1988)
7 Manabe S Bryan K & Spelman M J *J phys Oceanogr* (in the press)
8 Washington W & Meehl G *Clim Dynamics* **2**, 1-38 (1989)
9 Hansen J *et al J geophys Res* **93**, 9341-9364 (1988)
10 Wetherald R T & Manabe S *J atmos Sci* **45**, 1397-1415 (1988)
11 Bryan K & Lewis L J *J geophys Res* **84**, 2503-2517 (1979)
12 Ramanathan V R Cicerone R J Singh H B & Kiehl J T *J geophys Res* **90**, 5547-5566 (1985)
13 Bryan K *J phys Oceanogr* **14**, 666-673 (1984)
14 Manabe S & Stouffer R J *J Clim* **1**, 841-866 (1988)
15 Manabe S & Stouffer R J *J geophys Res* **85**, 5529-5524 (1980)
16 Manabe S & Bryan K *J Geophys Res* **90**, 11689-11707 (1985)
17 Broecker W S *Nature* **329**, 123-125 (1987)
18 Thompson S L & Schneider S H *Science* **217**, 1031-1033 (1982)
19 Deacon G E R *Discovery Rep* **15**, 125-152 (1937)
20 Sverdrup H V Johnson M W & Fleming R H *The Oceans* (Prentice Hall Englewood Cliffs 1942)
21 Semtner A J & Chervin R M *J geophys Res* **93**, 15502-15522 (1988)

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Cause of sulphate attack on concrete, render and stone indicated by sulphur isotope ratios

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SULPHATE attack is a significant problem which locally affects concrete and other construction materials in the United Kingdom and overseas. The mechanisms of sulphate attack are fairly well understood, but in some instances it has proved difficult to establish the origin of the sulphate Possible sulphate sources include soils and ground water, sea water, atmospheric pollution, contaminated ground-fill and highway de-icing salts Here we show that stable-sulphur-isotope analysis can help to identify the causes of sulphate attack We carried out this investigation primarily to determine whether sulphate impurities in rock salt used for winter maintenance of the roads could be responsible for higher than expected sulphate levels that have been observed in parts of some road bridges Our sulphur isotope results indicate that this is not the case, and indicate that atmospheric sulphate sources are more important

Sulphate attack can cause serious deterioration of hardened concrete, render and mortar[1-3] Both chemical and mechanical mechanisms may be involved In the presence of dissolved sulphates, the constituents of hardened Ordinary Portland Cement (OPC) paste are progressively chemically altered to form secondary minerals such as ettringite, thaumasite and gypsum[3 4] These changes can cause expansion, cracking and loss of cement-like characteristics and may ultimately lead to total failure of the structure The extent and rate of chemical alteration is dependent on the concentration and type of sulphate in solution Sulphate concentrations as low as 0 3 g l^{-1} can pose a significant threat to concrete made with OPC[5] Magnesium and ammonium sulphate solutions pose the greatest threat because they react chemically with all of the compounds present in hydrated OPC Sodium sulphate solutions only attack the tricalcium aluminate hydrate and calcium hydroxide components, whereas calcium sulphate solutions react only with tricalcium aluminate hydrate[1 2]

In some circumstances three physical mechanisms also contribute to the deterioration[6-8] (1) stress generated by salt crystallization from a saturated or supersaturated solution, (2) stress generated by thermal expansion of salt crystals, (3) stress caused when certain salts undergo an increase in volume during hydration The relative importance of these mechanisms is dependent on the nature of salts present, on fluctuations in ambient temperature and humidity, and on the physical characteristics of the material being attacked[7 8]

In assessing the likelihood or cause of sulphate attack it is therefore important to identify the type, concentration and origin of sulphates that may come in contact with the structure

Sulphate can be derived from several sources including naturally saline soils and ground water, sea water, atmospheric pollution, industrial effluents, ash and other solid wastes used as foundation material, oxidation of pyritic shales, oxidation of sulphide inclusions in concrete aggregates, highway de-icing salts, agricultural fertilizers and effluents, and bacterial degradation of sewage sludge[9-12] Chemical analysis of soils and ground waters, combined with petrographic examination of the concrete, normally allows some possible sulphate sources to be eliminated Here we show that stable-sulphur-isotope analysis can also assist in identifying the source of sulphate that has invaded concrete and related structures

The near-surface concrete in road bridges is quite frequently found to contain higher than expected levels of sulphate The cement in hardened OPC concrete normally contains 2 5–2 9% (average 2 7%) sulphate expressed as SO_3, but sulphate levels sometimes exceed 4 5% SO_3 by weight of cement in the outer parts of concrete structures that are 15–20 years old[13] On parts of the structures that are not in contact with soils or ground water, locally high sulphate levels might be explained by one of three factors (1) redistribution of sulphate originally present in the concrete by fluid migration, (2) ingress of sulphates present in de-icing salt solutions, (3) ingress of sulphur species from the atmosphere Here we identify the relative importance of these three possible sources in contributing to high sulphate levels that were found during an investigation of a number of Cambridgeshire bridges[13]

Most of the salt used for winter maintenance of the roads in the United Kingdom, including Cambridgeshire, is Triassic rock salt from the ICI Meadowbank mine at Winsford, Cheshire[14] This salt contains sulphate impurities mainly in the form of anhydrite ($CaSO_4$) Anhydrite is also the dominant sulphate impurity in de-icing salt from other sources used in the United Kingdom

To determine whether rock salt has contributed significantly to higher than expected sulphate levels found in the Cambridgeshire bridges studied, $\delta^{34}S$ values (defined in Table 1 legend) were determined for 44 samples of concrete, de-icing salt and other sulphate-bearing materials found in association with degraded concrete from a number of known environments A full list of the samples is given in Table 1

All of the samples were oven dried at 80 °C for 24 h, ground to pass a 0 1-mm sieve and boiled in 10% (v/v) HCl for 20 min to extract the sulphate After filtering, the acid-soluble sulphate was precipitated by addition of 5% (w/v) $BaCl_2$ solution The resulting $BaSO_4$ precipitates were then washed with de-ionized water and dried at 80 °C The $^{34}S/^{32}S$ ratios were determined by mass spectrometry performed on the SO_2 obtained by heating the $BaSO_4$ to ~1,400 °C (refs 15, 16) The overall reproducibility of a $BaSO_4$ standard was better than ±0 3%

The results showed the following (Fig 1) (1) samples of unhydrated OPC and pristine hardened concrete obtained from cores in Cambridgeshire bridges have $\delta^{34}S$ values of +10 to +14 5‰, (2) the samples of black ash, shale, clay and brick, which contain reduced sulphur compounds or oxidation prod-

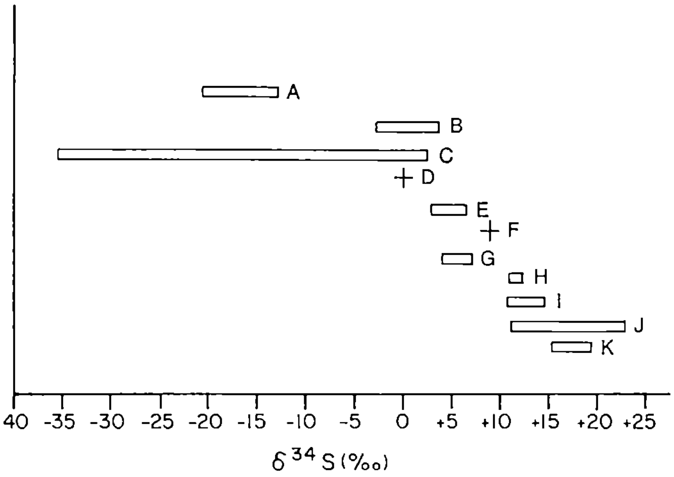

FIG 1 $\delta^{34}S$ values for A, brick (flettons), B, render affected by sulphate from brick/atmospheric sources, C, clay, shale and industrial ash ground-fill, D, concrete affected by sulphate from ash-fill, E, gypsum crusts on limestone buildings, F, sewer concrete affected by sulphuric trade effluent, G, deteriorated concrete, affected by atmospheric sulphate, H, pristine concrete, Peterborough, I, unhydrated OPC, J, other UK and overseas de-icing salt, K Cheshire de-icing salt

TABLE 1 Results of sulphur isotope analysis

Sample No	Nature of material	$\delta^{34}S$ (‰)*
Unhydrated cement		
KP3	Blue Circle OPC	+14.5
KP4	Rugby OPC	+10.6
Pristine hardened concrete from bridge cores		
KP13	depth 18 cm, Peterborough (Bretton)	+11.9
KP14	depth 18 cm, Peterborough South	+11.1
KP12	depth 15 cm, Peterborough (Fulbridge)	+11.2
Cheshire rock salt		
WIN1		+15.2
WIN2		+17.5
WIN3		+17.5
WIN4		+18.7
WIN5		+18.7
WIN6		+18.9
De-icing salt from other UK and overseas sources used in UK		
C1	sources confidential	+19.3
C2		+22.8
C3		+17.9
C4		+11.5
C5		+10.2
C6		+11.9
C7		+11.3
Brick		
KP33	Fletton KN	−20.4
KP34	Fletton BN	−14.9
KP35	Fletton CN	−13.3
Clay, shale and ash ground fill		
KP36	Oxford Clay, King's Dyke	−10.7
KP37	Oxford Clay, Calvert	+1.2
KP38	Oxford Clay, Bletchley	−1.8
KP31	oxidized Edale Shale, Carsington	−31.6
KP32	oxidized Edale Shale, Carsington	−36.0
KP29	black industrial ash, Birmingham	+2.6
KP30	red industrial ash Birmingham	−0.4
Stone weathering crusts		
KP23	gypsum crust on magnesian limestone, Westminster	+6.8
KP1	gypsum crust on limestone King's College, Cambridge	+4.0
KP2	gypsum crust on limestone, Pembroke College, Cambridge	+3.5
Deteriorated concrete and render		
KP10	deteriorated concrete affected by sulphates, Bretton Interchange, depth 0–5 cm, Peterborough	+5.7
KP25	as above	+7.1
KP11	deteriorated concrete affected by sulphates, Fulbridge footbridge deck, Peterborough, depth 0–5 cm	+6.9
KP27	calcite stalactite below soffit, Fulbridge footbridge	+4.7
KP24	carbonated concrete pillar protected from rainfall, underground car park, Huntingdon	+10.2
KP5	concrete paving slabs exposed to atmospheric sulphate but not treated with de-icing salt, Wolverhampton	+7.0
KP6	as above	+7.4
KP7	concrete cladding panels affected by atmospheric sulphate, 6th floor flat, Leeds	+5.9
KP8	as above	+6.9
KP26	sewer concrete attacked by sulphates and sulphuric acid in trade effluent, Cambridge	+8.7
KP28	concrete floor slab attacked by sulphate from black ash fill, Birmingham	0.0
KP9	render attacked by sulphates from brick, Cambridge University Computer building	−2.7
KP43	render attacked by sulphates, partly brick and partly atmospheric derived, Fowlmere	+3.8

* $\delta^{34}S = [(^{34}S/^{32}S)_{sample}/(^{34}S/^{32}S)_{standard}] - 1$, where we have used the Canyon Diablo Troilite (CDT) standard

ucts derived from them, have $\delta^{34}S$ values in the range −36 to +2.6‰, (3) concrete samples known to have suffered contamination by ingress of sulphates derived from the above sources have $\delta^{34}S$ values of −7 to +3.8‰, (4) gypsum crusts that have formed on the upper parts of limestone buildings in Cambridge because of the deposition of atmospheric sulphate have $\delta^{34}S$ values of +4 to +6.8‰, (5) deteriorated concrete from high levels on blocks of flats, which have also been affected by ingress of atmospheric sulphate, have $\delta^{34}S$ values of +5.9 to +7‰, (6) Cheshire rock salt supplied for winter maintenance of the roads has typical $\delta^{34}S$ values of +15.2 to +18.9‰, (7) samples of de-icing salt from a number of other mines in the United Kingdom and overseas, which are used less widely for winter maintenance, possess $\delta^{34}S$ values of +10.2 to +22.8‰, (8) samples of deteriorated concrete from bridges in the Peterborough area, which were found to have higher than expected sulphate levels but where the source of the extra sulphate was uncertain[13], has $\delta^{34}S$ values of +4.7 to +7.1‰.

These results clearly indicate that de-icing salt could not have contributed significantly to the elevated sulphate levels in the Cambridgeshire bridges investigated, because the rock salt has more positive $\delta^{34}S$ values, and the sulphate-contaminated concrete lower positive values, than the pristine hardened concrete, which has not been affected by ingress of external sulphate, at depths of 150–180 mm in these structures. The results also indicate that the high sulphate levels in the outer 40 mm of concrete cannot be attributed to redistribution, during wetting and drying, of sulphate initially present within the concrete itself. As the near-surface concrete with high sulphate levels has lower $\delta^{34}S$ values than the pristine concrete at greater depth, the extra sulphate is most likely to have been introduced from atmospheric sources. This may include both dry deposition of sulphur dioxide and wet deposition of sulphur species in rainfall and spray thrown up by vehicles on the carriageway.

We conclude that sulphur isotope analysis can provide a useful tool in investigations to determine the causes of sulphate attack. Further work is required to determine whether atmospheric sulphur deposited by wet and dry processes can be differentiated and whether the incorporation of external sulphate in stone crusts and concrete involves any significant differences in isotope fractionation. □

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1. Figg, J. *Chem. Ind.* 17 October, 770–775 (1983).
2. Benstead, J. *World Cement Technol.* May, 178–184 (1981).
3. Mehta, P. K. *Cement Concrete Res.* **13,** 401–406 (1983).
4. Crammond, N. J. *Cement Concrete Res.* **15,** 431–441 (1985).
5. BRE Digest **250** *Concrete in Sulphate-bearing Soils and Groundwaters* (Building Research Establishment, Garston, 1981).
6. Cooke, R. U. & Smalley, I. J. *Nature* **220,** 1226–1227 (1968).
7. Sperling, C. H. B. & Cooke, R. U. *Earth Surface Processes and Landforms* **10,** 541–555 (1985).
8. Goudie, A. S. *Sch. Geogr. Res. Pap.* **33,** (University of Oxford, 1986).
9. Lossing, F. A. *Highway Res. Board Rec.* **113,** 88–103 (1966).
10. Moum, J. & Rosenquist, I. T. *J. Am. Concrete Inst.* September, 257–264 (1959).
11. Cheetham, D. W. & Banfill, P. F. G. *Construction Pap.* **1,** 7–16 (1980).
12. St John, D. A. *Cement Concrete Res.* **12,** 633–639 (1982).
13. Pye, K. & Hudson, A. J. *New Civil Engnr* 10 July, 16–17 (1986).
14. Mineral Resources Consultative Committee *Salt.* (HMSO, London, 1971).
15. Holt, B. D. & Engelkemeir, A. G. *Analyt. Chem.* **42,** 1451–53 (1970).
16. Faure, H. *Principles of Isotope Geochemistry* 2nd edn 523–552 (Wiley, New York, 1986).

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Thermal control of basaltic fissure eruptions

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As hot, basaltic magma rises in a newly opened dyke during an eruption, it flows through colder crustal rock. The flowing magma advects heat along the dyke, at the same time, heat is conducted out of the dyke into the colder surroundings. The loss of heat will lead initially to the channel becoming constricted by magma solidifying against the walls The channel may then become completely blocked, which will end the eruption at that site before the supply of magma is exhausted Alternatively, the continual supply of heat by the flowing magma may, after some time, exceed the losses into the country rock. In this case, the initial solidification is reversed, the walls of the channel are progressively melted and the dyke is widened until the supply diminishes We present here a model that quantitatively delineates these two regimes. We also identify an intermediate regime in which parts of the surface fissure may become blocked and the eruption continues from isolated vents

Basaltic eruptions display a fascinating variety of behaviours[1] The low viscosity of such lavas allows relatively steady degassing and flow, as opposed to the explosive eruptions characteristic of more viscous magmas 'Quiet' basaltic eruptions nonetheless evolve over time in a highly complex way Typically, a linear system of fissures opens rapidly and erupts a continuous 'curtain of fire' Small eruptions simply cease within half a day In larger eruptions activity begins, after several hours, to concentrate at certain points along the fissure[2,3] If eruption persists, it becomes localized at one or a few vents, building cones Preferential localization of subsequent fissure eruptions to the same areas produces substantial shield volcanoes In the extremely voluminous eruption at Laki in 1783, flow persisted at not one but dozens of vents along the fissure[4]

The initial formation of the dyke is the result of elastic processes in the surrounding country rock[5 6] We concentrate here on the primarily thermodynamic problem of determining both the flow in the dyke and its evolution after the initial rock propagation and widening have taken place Naive estimates of the solidification of magma against the walls of the dyke based solely on conduction[7], indicate that flow should effectively cease in a matter of days A previous model[8] which included some advective effects came to much the same conclusion unless brecciation of the dyke wall and local widening of the conduit by removal of the fractured blocks leads to increased flow rates[9] There are deficiencies in this model, however, most notably the neglect of both the latent heat of solidification and the effect of the thermal advection in the magma on the temperature profile of the solid These omissions consistently overestimate the tendency of the dyke to become blocked Our model includes both these effects and allows us to understand how meltback of the walls can actually occur

To obtain our results we analysed an initial-value problem for $\theta(x, z, t)$, the temperature in both the fluid and the solid with respect to locally cartesian coordinates z measured along the wall and x perpendicular to it, with $x=0$ at the wall In constructing the equations, we assumed that the flow in the thermal boundary can be represented by a shear flow of magnitude γ The governing equations then become

$$\theta_t - v\theta_x + \gamma x \theta_z = k\theta_{xx} \ (x, t>0) \tag{1}$$

$$\theta = T_m \ (z=0), \qquad \theta = T_w \ (x=0), \qquad \theta = T_m \ (t=0 \text{ or } x \to \infty) \tag{2}$$

$$\theta_t - v\theta_x = k\theta_{xx} \ (x<0, t>0) \tag{3}$$

$$\theta = T_w \ (x=0), \qquad \theta = T_\infty \ (t=0 \text{ or } x \to -\infty) \tag{4}$$

The velocity of migration of the interface between fluid and solid, v is proportional to the difference in the conductive heat flux across the wall and is determined from

$$(L/c)v = -\kappa[\theta_x(0+, z, t) - \theta_x(0-, z, t)] \tag{5}$$

The shear flow at the edges of the channel of width W is driven by a pressure difference ΔP and results in a volume flow rate $Q(t)$ These are related by

$$\Delta P = 12\mu Q(t) \int_0^h W^{-3}(z, t)\,\mathrm{d}z \quad \text{and} \quad \gamma(z, t) = 6W^{-2}(z, t)Q(t) \tag{6}$$

With either solidification or melting, the half-width gradually changes according to

$$W(z, t) = W_i - 2\int_0^t v(z, t')\,\mathrm{d}t' \tag{7}$$

The solutions to equations (1)-(7) were obtained numerically by integrating suitably simplified and non-dimensionalized versions which are formally valid for all time[10 11]

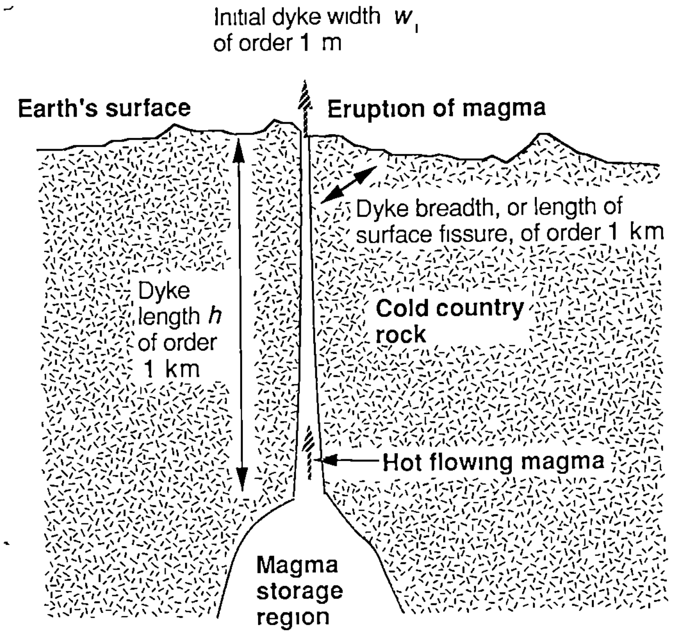
Initial dyke width w_i of order 1 m
Earth's surface
Eruption of magma
Dyke breadth, or length of surface fissure, of order 1 km
Dyke length h of order 1 km
Cold country rock
Hot flowing magma
Magma storage region

FIG 1 Cross-section through a typical sheet-like planar dyke Note that the dyke need not necessarily be vertical

We consider, as depicted in Fig 1, the dyke to be a two-dimensional channel the width of which is initially uniform but subsequently changes because of local solidification or melting The newtonian flow is considered to be laminar and the driving pressure is taken to be fixed Thus if the channel becomes constricted, the total flow rate decreases Cooling of the magma in the dyke is typically confined to a thin boundary layer adjacent to each wall The width of the boundary layer increases with length along the dyke and can attain a value of ~10 cm at the end of the dyke The difference between the heat supplied to the wall from this boundary layer and that conducted into the surrounding solid determines the rate of solidification or melting, which is directly proportional to the magnitude of the latent heat The temperature profile in the solid is determined numerically taking into account the effects of advection in the magma A full derivation of the governing equations and details of their solution are given in refs 10 and 11

The behaviour of the solutions is governed by three dimensionless parameters The first is the Stefan number of the magma, $S_m = L/(c(T_m - T_w))$, where L and c are the latent and specific heats per unit mass, T_m is the temperature of the magma at the entrance to the dyke and T_w is the temperature at the wall, which is defined as the temperature at which the crystallizing magma ceases to flow In fact, solidification and melting take place over a temperature range, this range is, however, typically small compared with the difference between T_w and T_∞, the far-field temperature in the crust, and hence can be neglected The second parameter governing the behaviour of the thermal processes is the Stefan number of the surrounding crust $S_\infty = L/(c(T_\mathrm{w} - T_\infty))$ This incorporates the approximation that the fluid magma, its solid product and the country rock have identical thermal properties The final parameter is $B = (W_\mathrm{i}^4/H^2)(\Delta P/\mu\kappa)$, where W_i is the initial width of the dyke of length H, ΔP is the driving pressure in the reservoir, μ is the mean value of the dynamic viscosity of the magma within the boundary layer and κ is the thermal diffusivity of both magma and country rock The cube root of B is the ratio of the initial width of the dyke to the initial width, at the top of the dyke, of the thermal boundary layer, due to advection before any solidification or melting can take place We assume that this ratio is large

We also assume that $c = 730\,\mathrm{J\,kg^{-1}\,°C^{-1}}$, $L = 8\times10^5\,\mathrm{J\,kg^{-1}}$, $\kappa = 10^{-6}\,\mathrm{m^2\,s^{-1}}$, $T_\mathrm{m} = 1{,}200\,°\mathrm{C}$, $T_\mathrm{w} = 1{,}150\,°\mathrm{C}$ and $\mu = 100$ Pa s (ref 12), which reflect values appropriate for a typical basaltic magma The driving pressure was evaluated from the lithostatic overburden[13] as $\Delta P/H = 2{,}000\,\mathrm{N\,m^{-3}}$, with H taken as either 2 or 5 km We chose these values because the depth interval 2-5 km corresponds to the top and bottom of active magma reservoirs in Hawaii, Iceland and the East Pacific Rise It also corresponds approximately to the upper surface and the keel of dykes in the respective rift zones of these localities In a given volcanic system the remaining two variables T_∞ and W_i determine the course of successive eruptions The value of T_∞ may vary between 0 and 1,150 °C (T_w) depending on the previous thermal history of the dyking zone With these values, $S_\mathrm{m} = 22$, S_∞ ranges upwards from 1 05 (which corresponds to cold country rock at 0 °C) and B can take on any positive value, though it must exceed unity for our boundary-layer model to be appropriate

The important qualitative behaviour of our two-dimensional model is sketched in Fig 2 This is augmented by the quantitative results depicted in Fig 3 When the eruption commences magma initially solidifies against the walls of the dyke In those dykes for which W_i and T_∞ are sufficiently small so that they fall below demarcation line AA′ for a 2-km dyke and BB′ for a 5-km dyke, solidification continues in the downstream portion of the channel The surface fissure will eventually become blocked and the eruption through the dyke feeding the fissure will cease irrespective of the magma supply If $T_\infty = 100\,°\mathrm{C}$, for a dyke 2 km long the critical initial width is 1 3 m, whereas for a dyke 5 km long the critical initial width is 1 7 m The time taken for the dyke to become blocked as a function of its initial width is shown in Fig 3*b* for these two dyke lengths

If ΔP remains fixed, eruptions can only continue from dykes whose initial width exceeds the critical value, which is decreased if the dyke is surrounded by warmer country rock This increase in T_∞ could be the result of pre-warming by previous dyking events or by hydrothermal circulation For dyke widths beyond the critical value the thermal energy from the reservoir advected along the dyke melts back the walls and the channel remains open Thereafter, the rate and duration of the eruption are controlled by the decay of the driving pressure ΔP and the possible closing of the dyke because of the resulting subsidence, which we have not included in our model When ΔP has decreased sufficiently solidification will take place once more and the eruption fade away

The concept of the two-dimensional widening of a dyke as a

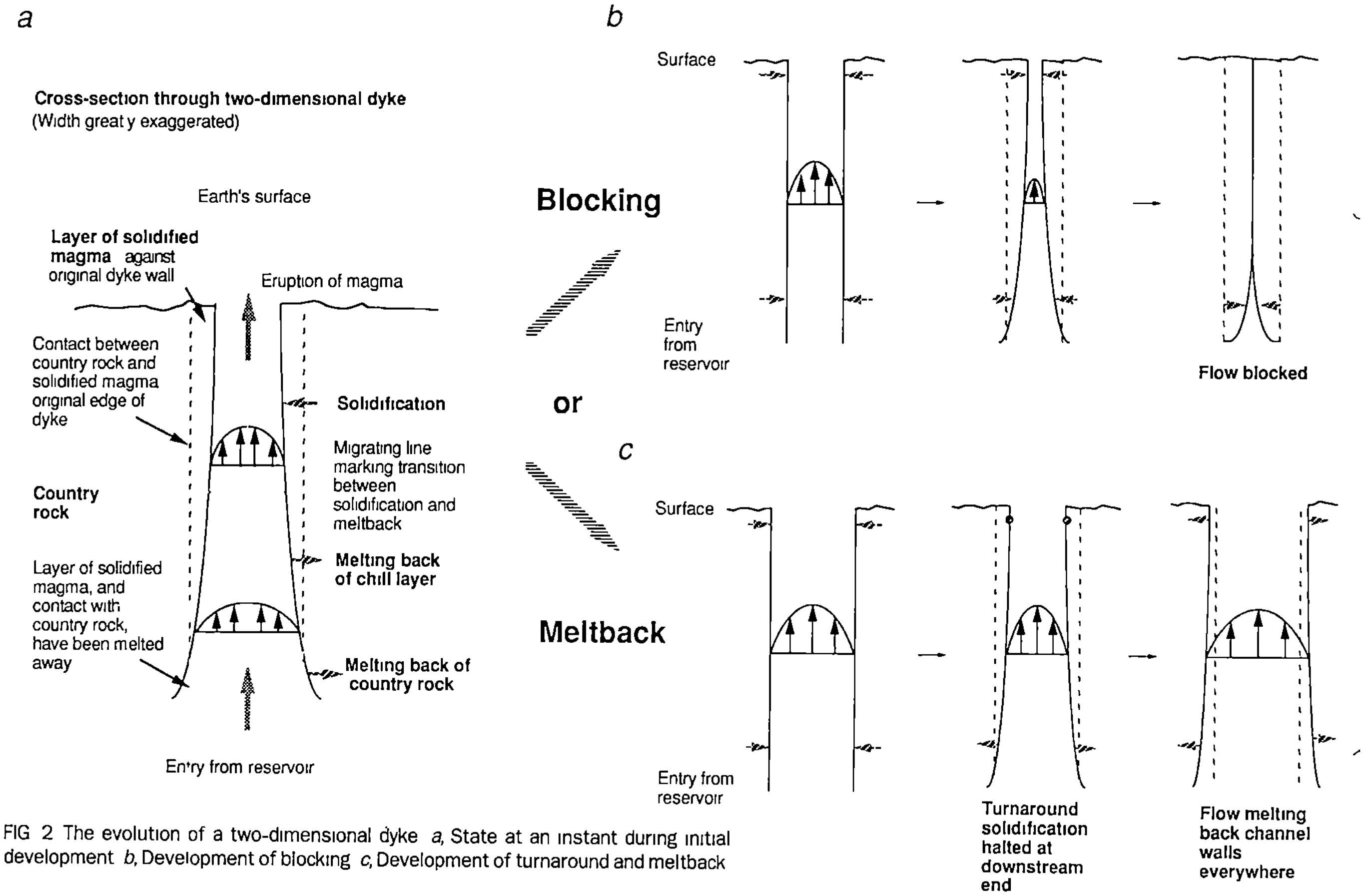
a
Cross-section through two-dimensional dyke
(Width greatly exaggerated)
Earth's surface
Layer of solidified magma against original dyke wall
Eruption of magma
Contact between country rock and solidified magma original edge of dyke
Solidification
Migrating line marking transition between solidification and meltback
Country rock
Melting back of chill layer
Layer of solidified magma, and contact with country rock, have been melted away
Melting back of country rock
Entry from reservoir
b
Surface
Blocking
Entry from reservoir
Flow blocked
or
c
Surface
Meltback
Entry from reservoir
Turnaround solidification halted at downstream end
Flow melting back channel walls everywhere

FIG 2 The evolution of a two-dimensional dyke *a*, State at an instant during initial development *b*, Development of blocking *c*, Development of turnaround and meltback

result of melting is broadly consistent with some previous observations Both the evolution of the Pu'u O'o series of en echelon dyke fissures in Hawaii (L Wilson, personal communication) and the final width of 25 m for some fissure dykes in Iceland (M R Ryan, personal communication) seem to be explicable only if melting back of the original dyke walls is postulated

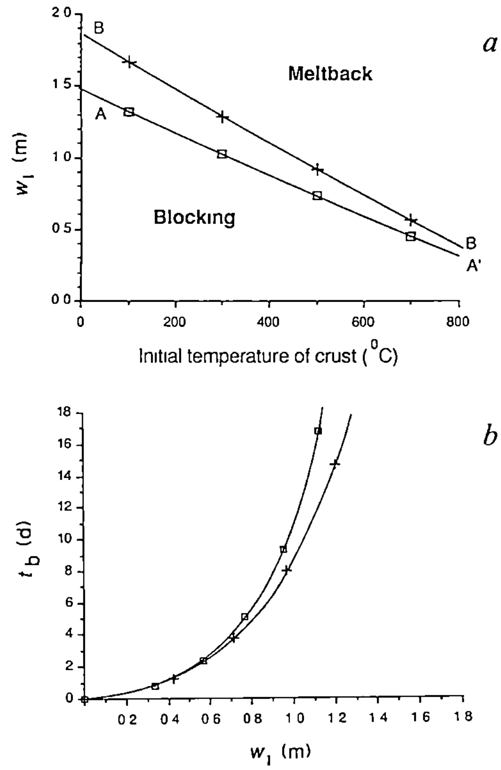

FIG 3 Numerical results of model, for parameter values given in text *a*, Regions of parameter space in which blocking or meltback are predicted for dyke length 2 km (squares) and 5 km (crosses) *b*, Time for dyke to become blocked as a function of initial dyke width, for $T_\infty = 100$ °C The time to block becomes infinite for widths approaching the critical value for meltback to occur (dotted lines (left) $H = 2$ km, dotted lines (right) $H = 5$ km) Symbols as in *a*

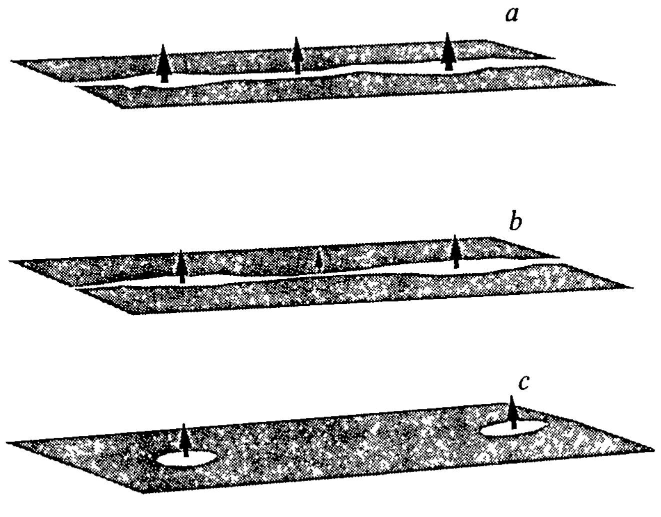

FIG 4 A schematic (not to scale) diagram of flow localization and development of a surface fissure over time *a*, initial irregularities of width (which may be small) *b*, faster solidification in narrow regions exaggerates the difference *c*, flow through isolated vents melts back walls and flow is blocked elsewhere

Further, the two-dimensional analysis quantifies an important feedback mechanism Decreasing the width of the dyke restricts the flow This reduces the advected heat supply which leads to further solidification and reduction of the width Correspondingly, widening the dyke by melting the walls increases the advected heat supply which results in further melting This feedback mechanism has an important role in a more realistic three-dimensional model, in which the width of the dyke varies, as depicted in Fig 4 Cross-flows between sections of different initial widths will form because flow through the narrow sections will encounter greater constrictions because of preferential solidification The cross-flows further reduce the advected heat flux in the downstream portions of the narrow sections and increase it at the wider portions, thereby exaggerating initially small differences in the width of the dyke (Fig 4*b*) Our model predicts that, as a result of this mechanism, many fissure eruptions of moderate size are localized at isolated vents over a period of days (Fig 4*c*), which is consistent with observations on Hawaii and elsewhere (refs 2, 3, 14 and T L Wright, personal communication) and with a previous qualitative discussion[15] Eruptions from these vents will persist until limited by other volcanic processes Sustained eruptions result in the formation of cylindrical conduits that are much wider than the original dyke and are preserved as volcanic plugs ☐

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1 Macdonald G A & Abbot A T *Volcanoes in the Sea* (University of Hawaii Press Honolulu 1970)
2 Richter D H Eaton J P Murata K J Ault W A & Krivoy H L *US Geol Surv Prof Pap* 537 E (1970)
3 Thorarinsson S Steinthorsson S Einarsson T H Kristmannsdottir H & Oskarsson N *Nature* **241**, 372-375 (1973)
4 Thorarinsson S *Bull Vol* **33**, 910-929 (1969)
5 Jaeger J C *Basalts The Poldervaart Treatise on Rocks of Basaltic Composition* (eds Hess H H & Poldervaart A) 503-536 (Interscience New York 1968)
6 Spence D A & Turcotte D L *J geophys Res* **90** 575-580 (1985)
7 Lister J R *J Fluid Mech* **210** 263-280 (1990)
8 Delaney P T & Pollard D D *Am J Sci* **282** 856-885 (1982)
9 Delaney P T & Pollard D D *US Geol S Prof Pap* 1202 (1981)
10 Bruce P M thesis Univ of Cambridge (1989)
11 Bruce P M & Huppert H E in *Magma Transport and Storage* (ed Ryan M P) (Wiley London in the press)
12 Huppert H E & Sparks R S J *Contr Miner Petrol* **75**, 279-289 (1980)
13 Wilson L & Head J W *J geophys Res* **86** 2971-3001 (1981)
14 Anderson I *New Scientist* **1567**, 50-54 (1987)
15 McBirney A R *Igneous Petrology* (Freeman-Cooper San Francisco 1984)

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Hydrothermal plumes along the North Fiji Basin spreading axis

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STEADY-STATE emanations of hydrothermal vent fluid along the mid-ocean ridges give rise to plumes rising 100–300 m above the sea floor, identified by anomalies of temperature, conductivity, chemistry and particles Recently, a quite different type of hydrothermal plume created by a brief but massive release of high-temperature hydrothermal fluids was discovered by Baker *et al.* at a Juan de Fuca vent field[1–3]. This gigantic thermal plume was termed a 'megaplume'. In December 1987, we found a large

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hydrothermal plume showing anomalies of temperature, methane and manganese above the North Fiji Basin spreading axis The transience of this plume at 173° 30′ E, 18° 50′ S was confirmed by our return visit to the site in November 1988 The discovery of megaplumes at two sites in very different tectonic environments indicates that this type of hydrothermal activity may make an important contribution to thermal and chemical fluxes from the mid-ocean ridges.

The North Fiji Basin is a 2,000–4,000-m-deep back-arc basin behind the active subduction zone of the Vanuatu Arc (Fig 1*a*) with a spreading rate[4] of ~7 2 cm yr^{-1} In December 1987, the 'Kaiyo 87' cruise was conducted (the STARMER project[5]) to locate the recent spreading axis more precisely[6] The cruise followed up the results of a previous survey[4] (SEAPSO leg 3, December 1985) Here we describe the results of conductivity-temperature-depth (CTD) profiling and water sampling and discuss the nature of the plumes observed

Because no precise vent locations had been discovered, we used eleven hydrocast stations along the ridge crest (Fig 1*b*) The background concentrations of Mn and CH_4 ranged from 0 4 to 1 0 nmol kg^{-1} and from 0 2 to 0 3 nmol kg^{-1}, respectively These are comparable with the background levels for deep waters of the open oceans[7,8] We observed Mn anomalies at all stations the Mn concentration increased with depth below 1,900 or 2,200 m The Mn concentrations of the bottom layer at the nine stations, located between 16° 54′ S and 18° 50′ S, ranged between 5 and 10 nmol kg^{-1} This indicates that the hydrothermal supply of Mn extends over a large area similar to the Juan de Fuca Ridge[9] The anomalies of Mn at the northernmost and the southernmost stations (stations 11 and 15) were the smallest

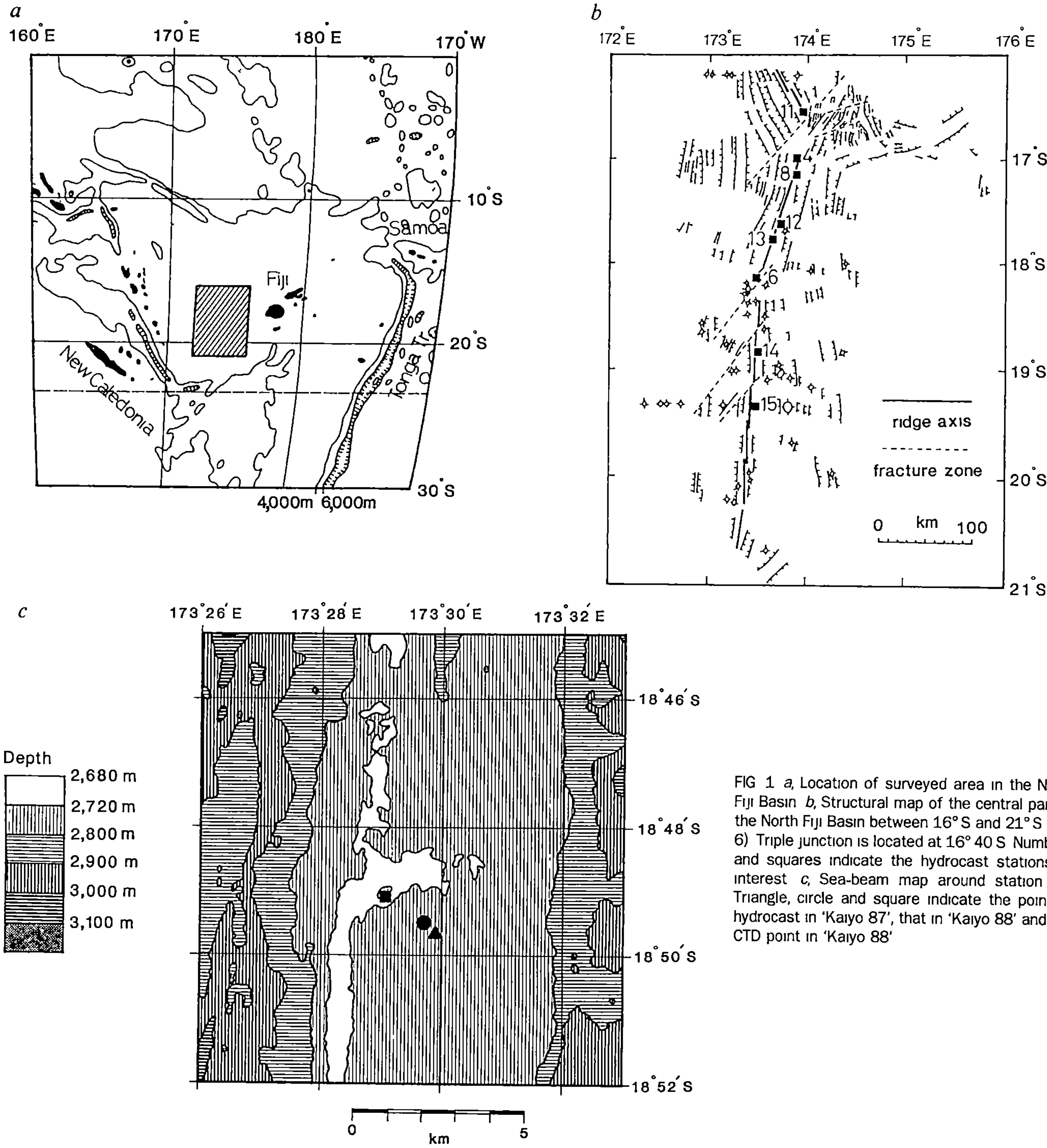

FIG 1 *a*, Location of surveyed area in the North Fiji Basin *b*, Structural map of the central part of the North Fiji Basin between 16° S and 21° S (ref 6) Triple junction is located at 16° 40 S Numbers and squares indicate the hydrocast stations of interest *c*, Sea-beam map around station 14 Triangle, circle and square indicate the point of hydrocast in 'Kaiyo 87', that in 'Kaiyo 88' and the CTD point in 'Kaiyo 88'

TABLE 1 Estimated flux ratios of heat, Mn and CH_4 in hydrothermal solutions

Locations	Mn/heat (nmol cal^{-1})	CH_4/heat (nmol cal^{-1})	CH_4/Mn (mol mol^{-1})	References
EPR, 21° N, vent water	2-2 8	—	—	11
EPR, 20° S, VULCAN, large plume	0 3	—	—	7
EPR, 20° S, VULCAN, small plume	>1	—	—	7
Southern JF, plume	—	—	0 09	12
JF, SSS, vent water	7 6-15 7	—	—	13
JF, SSS, plume	1 4	—	—	14
JF, SSS, megaplume	0 37	—	—	2
JF, SSS, steady-state plume	3 5	—	—	2
JF, Endeavour, plume	0 4-1 0	6	6-16	15
Okinawa Trough, Iheya Glaben	—	—	0 6-3 2	16
NFB, SEAPSO, average deep water	—	—	0 12	4
NFB, SEAPSO, high CH_4 plume	—	—	0 24-0 31	4
NFB, Kaiyo 87, high Mn plume	0 92	0 05	0 056	This work
NFB, Kaiyo 87, average deep water	—	—	0 121	This work
NFB, Kaiyo 87, high CH_4 plume	0 13	0 05	0 386	This work

EPR, East Pacific Rise, JF, Juan de Fuca, NFB, North Fiji Basin
SSS, southern symmetrical segment

the maximum concentrations being $<5\ \mathrm{nmol\ kg^{-1}}$

The plot of potential temperature θ against salinity S for the station 14 (triangle in Fig 1*c*, 173° 30′ E, 18° 50′ S, 29 December 1987) data is shown in Fig 2*a* The apparent distortion reflects the θ-anomaly that is due to the injection of hydrothermal water into the ambient sea water Figure 2*b* is the calculated profile of the excess temperature It shows a large plume, having a sharp peak 800 m above the sea floor and a broad peak 650 m above the sea floor There is another deeper plume 90 m above the sea floor The density-depth profile shows no inversion so these are neutrally buoyant plumes spreading along the isodensity surface The physical characteristics of the Juan de Fuca Ridge megaplume[2,3] are as follows to more than 800 m above the sea floor it is 700 m thick, has a 20-km-diameter spheroidal shape and a maximum θ-anomaly of 0 28 °C Beneath the megaplume, a separate deeper plume was observed The vertical scale and the θ-anomaly of the upper plume of station 14 are similar to those of the Juan de Fuca Ridge megaplume The deeper plume corresponds to the steady-state plume of the Juan de Fuca Ridge Smaller θ-anomalies were observed at station 8 and 13 At the other stations, smooth θ-S relationships were obtained

Figure 3 displays the Mn and CH_4 profiles at station 14 The upper large plume in 'Kaiyo 87' is characterized by a low Mn concentration but the deeper narrow plume shows a high Mn concentration The shapes of the vertical profiles of Mn in both the Juan de Fuca Ridge and the North Fiji Basin are very similar but the Juan de Fuca Ridge plumes had concentrations about seven times higher than that of the North Fiji Basin

The correlation of Mn and CH_4 concentrations for station 14 is plotted in Fig 4 along with those at station 12 in 'Kaiyo 87' At station 12, we found no θ-anomaly but the concentration of Mn increased gradually with depth below 2,200 m The linear CH_4/Mn relationship may represent an average injection ratio of CH_4 and Mn in the area The slope (0 121 mol mol^{-1}, regression line B in Fig 4) is the same as the averaged CH_4/Mn ratio (0 12 mol mol^{-1}) in the previous SEAPSO results[4] (Table 1)

The available data for the hydrothermal-supply ratios of CH_4/heat, Mn/heat and CH_4/Mn are summarized in Table 1 Regression line A in Fig 4 corresponds to the upper plume of station 14, which has a high CH_4/Mn ratio The regression line C corresponds to the deeper plume of this station, which has a low CH_4/Mn ratio We calculated the Mn/heat and CH_4/heat ratios for both plumes The relationship of Mn/heat ratio between the upper and lower plumes of station 14 corresponds to that between the megaplume and the accompanying steady-state plume of the Juan de Fuca Ridge[2,3]

We made a return visit to station 14 on 20 November 1988 during the 'Kaiyo 88' cruise (circle in Fig 1*c*) The distance between the two sampling points was only a few hundred metres, but we found no θ-anomaly The Mn profile in 'Kaiyo 88' is shown in Fig 3 (closed circles) The upper plume of the previous year had disappeared but a large Mn anomaly was observed near the sea floor We detected a small θ-anomaly of 0 02 °C, with a profile similar to the lower plume of the previous year, at a nearby CTD station (square in Fig 1*c*) We also detected vent animal communities (deep-tow camera observation) during 'Kaiyo 88' These observations indicate hydrothermal activity in the area It is likely that the 'Kaiyo 88' hydrocast detected

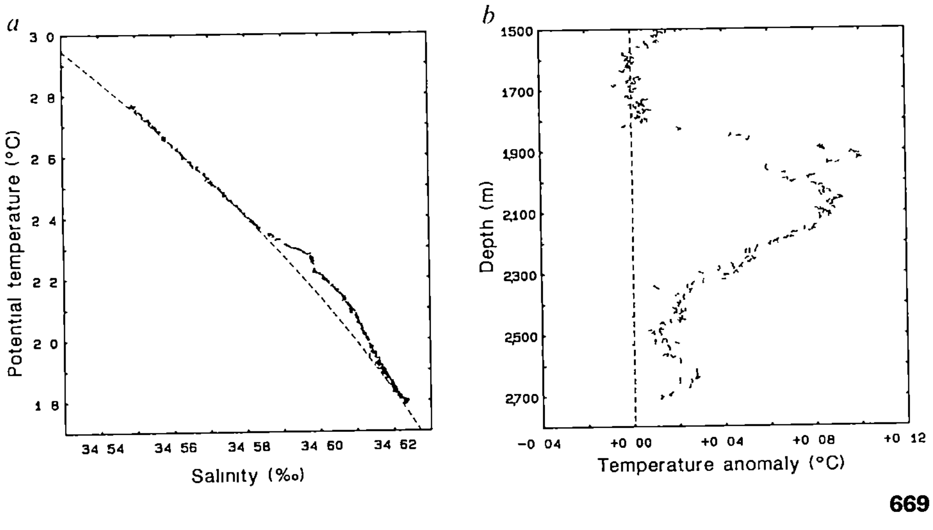

FIG 2 *a*, Potential temperature (θ) plotted against salinity (S) at station 14 of 'Kaiyo 87' Seabird model 9 CTD with rosette sampler was used The resolution of the thermometer and conductivity cell were 0 0003 °C and 0 00004 S m^{-1}, respectively Data obtained by CTD was averaged in every 1-m interval for water-column depths >1,500 m The broken line is the smoothed curve obtained from the θ-S relationship at station 15, the nearest station having no temperature anomaly to station 14 *b*, Vertical profile of excess temperature in the water column of station 14 of 'Kaiyo 87', calculated as a difference between the potential temperatures at stations 14 and 15 at the same salinity

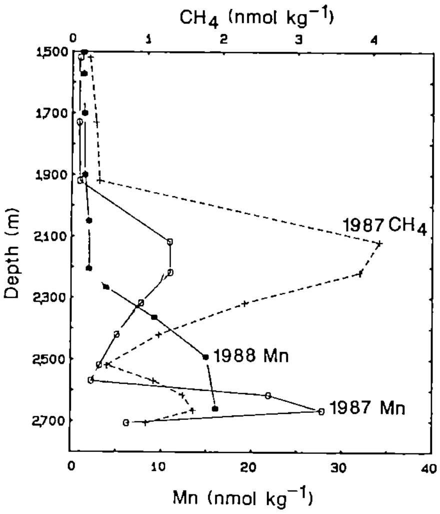

FIG 3 *a*, Vertical profiles of Mn and CH_4 at station 14 Open circles, crosses and closed circles are Mn of 'Kaiyo 87', CH_4 of 'Kaiyo 87' and Mn of 'Kaiyo 88', respectively Water samples were collected with Teflon-lined 8 l Go-Flo Niskin bottles mounted on rosette multi-sampler Mn in sea water was collected aboard ship on a Nucleopore membrane filter with added ferric hydroxide precipitate Mn in both dissolved and particulate forms are effectively separated from the seawater matrix The precipitate was dissolved in hydrochloric acid and analysed by inductively-coupled plasma emission spectrometry on shore Samples for CH_4 analysis were sealed in glass bottles with septum stopper after poisoning with $HgCl_2$ and preserved in a refrigerator until they were analysed on shore[16] The limit of determination of Mn and CH_4 analyses were both 0 1 nmol kg^{-1} The reproducibility of Mn analysis was 2% at 7 nmol kg^{-1}, but 10% at 1 nmol kg^{-1} CH_4 analysis was accurate within 4% at 1 nmol kg^{-1}

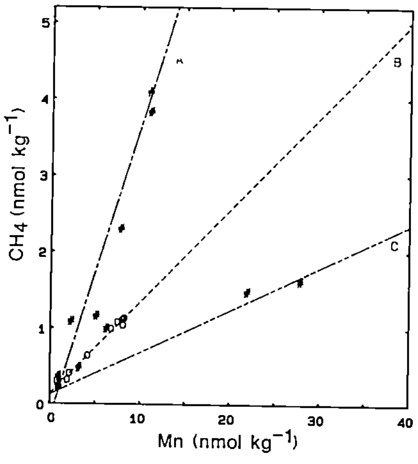

FIG 4 Correlation between Mn and CH_4 concentrations in sampled sea water of stations 12(o) and 14(#) of 'Kaiyo 87' cruise Regression lines were calculated for the data of stations 8, 12, 13 and 14 A, CH_4(nmol kg^{-1})= 0 386 ×Mn(nmol kg^{-1})−0 16 (correlation coefficient, r=0 90, number of points, n=9), B, CH_4(nmol kg^{-1})=0 121 ×Mn(nmol kg^{-1})+0 14 (r=0 99, n=11), C, CH_4(nmol kg^{-1})=0 056 ×Mn(nmol kg^{-1})+0 13 (r=0 93, n=9)

the steady-state hydrothermal effluent emerging at the spreading axis and that the upper plume of the previous year had disappeared

The identification of the upper plume of the 'Kaiyo 87' station 14 as a megaplume depends on its vertical scale, its large θ-anomaly, the low concentration of Mn and its absence in 'Kaiyo 88' These characteristics are similar to those of the Juan de Fuca Ridge megaplume During an earlier cruise, Craig *et al*[10] discovered a large plume having a very elevated concentration (13 nmol kg^{-1}) of CH_4 in the North Fiji Basin Our hydrocast at the location (station 6 in Fig 1*b*) revealed no plume-shaped anomaly of Mn and the CTD profile showed no temperature anomaly The CH_4 plume observed by Craig *et a* may have been another megaplume

The different Mn/heat ratio of the upper plume and lowe plume of station 14 could be a consequence of a different origi The extraction efficiency of Mn into vent fluid would depen on the reaction time of the fluid with hot basalt and the effectiv water/basalt ratio during plume formation The sudden catas trophic production of a megaplume might thus be expected t result in a low Mn/heat ratio compared with a steady-stat plume

The observations of megaplume-type hydrothermal effluen at different ocean ridges indicates that this type of hydrotherma activity may be a common phenomenon in rift systems Th possibility of a frequent occurrence of megaplumes is significan for the global hydrothermal fluxes of heat and chemical com ponents More extensive investigations of megaplumes especially of their frequency, spatial scale and chemical nature are necessary □

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1 Cann J & Strens R *Nature* **329**, 104 (1987)
2 Baker E T Massoth F J & Feely R A *Nature* **329**, 149–151 (1987)
3 Lupton J E Baker E T & Massoth G J *Nature* **337**, 161–164 (1989)
4 Auzende J M *et al Tectonophysics* **146**, 317–351 (1988)
5 Swinbanks D *Nature* **328**, 4 (1987)
6 Auzende J M *et al C r hebd Séanc Acad Sci Paris, Ser* 2 **306**, 971–978 (1988)
7 Klinkhammer G & Hudson A *Earth planet Sci Lett* **79**, 241–249 (1986)
8 Charlou J L Rona P & Bougault H *J mar Res* **45**, 461–472 (1987)
9 Jones C J Johnson H P & Delaney J R *Geophys Res Lett* **8**, 873–876 (1981)
10 Craig H Craig, V K & Kim K R *Eos* **68**, 100–101 (1987)
11 Von Damm K L *et al Geochim cosmochim Acta* **49**, 2197–2220 (1985)
12 Winn C D Karl D M & Massoth G J *Nature* **320**, 744–746 (1986)
13 Von Damm K L & Bischoff J L *J geophys Res* **92**, 11334–11346 (1987)
14 Baker T E & Massoth G J *Science* **234** 980-982 (1986)
15 Rosenberg N D *et al Nature* **334**, 604–607 (1988)
16 Ishibashi J *et al Geochem J* **22**, 107–114 (1988)

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An unusual hopane biodegradation sequence in tar sands from the Pt Arena (Monterey) Formation

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HOPANES are ubiquitous in petroleum source rocks and crude oils These compounds are thought to be derived mainly from a specific oxygenated precursor, bacteriohopanetetrol, found in the cell walls of prokaryotic organisms[1], and commonly occur as a homologous series of structurally related isomers containing between 27 and 35 carbon atoms; hopanes containing more than 35 carbon atoms have also been reported[2]. Hopanes and other pentacyclic triterpanes are relatively resistant to microbial degradation[3–5], making them useful in correlating moderately biodegraded oils to their source rocks[5]. During the advanced stages of biodegradation, however, these compounds can be altered, being converted either to 25-normethyl homologues[4] or to other, as yet uncharacterized, products. Selective removal of individual compounds may occur; for example, 22*R* extended homohopanes may be degraded whereas 22*S* epimers are preserved[6–8]. Here we report an unusual biodegradation sequence of pentacyclic triterpanes in tar sands from the Pt Arena Formation, California, in which the 22*S* and 22*R* isomers of the 17α(H), 21β(H)-C_{35} extended hopanes are selectively preserved. Demethylation at the C_{25} position is observed for most hopanes in severely biodegraded samples, with

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the exception of the C_{35} extended hopanes Two commonly occurring trisnorhopanes[9], designated Ts (18α(H), 21β(H)-22, 29, 30-trisnorneohopane) and Tm (17α(H), 21β(H)-22, 29, 30-trisnorhopane), also seem resistant to degradation These findings, together with previous studies of crude-oil biodegradation, imply that biodegradative alteration of hopanes can proceed through numerous pathways, which can result in widely differing distributions

We have analysed several tar-sand samples from the Miocene Pt Arena (Monterey) Formation in the Pt Arena Basin, California Tar-stained sandstones are distributed throughout siliceous organic mudstones exposed at several locations along the northern California coastline The thickest continuous tar-sand unit measures ~15 m, and consists of graded, medium-coarse-grained sands containing mudstone clasts at the base of some individual beds We collected the samples near this unit, either directly from the exposure or from eroded fragments lying nearby All samples seemed heavily weathered and varied in composition from a pliable tar distributed throughout a sand matrix to more bituminous, less viscous samples which would flow upon heating by sunlight We assume that most, if not all, biodegradation in these tar sands occurred following their exposure

Methylene chloride extracts of the tar sands consist of a sulphur-rich, bituminous material enriched in aromatic and polar components The composition of C_{15+} solvent-extractable material in four samples is shown in Table 1 Saturated hydrocarbons make up a small fraction of the extract (3 7–11 1%) and the asphaltene contents vary from 16 5 to 54 1% The sulphur content of the extractable material varies from 3 2 to 5 8% (Table 1) These characteristics are typical of high-sulphur petroleums, such as those in the Monterey Formation[10], that have been severely biodegraded Isotopically heavy ^{13}C compositions for the saturated and aromatic hydrocarbons (Table 1) indicate that the tar was most likely generated from facies equivalent to the Monterey Formation in the Pt Arena Basin[10 11]

The saturated hydrocarbons isolated from the four samples consist primarily of naphthenic compounds and are devoid of *n*-alkanes and isoprenoids, a composition characteristic of moderate to severe biodegradation[5,12] We confirmed the presence of tricyclic and pentacyclic triterpanes by gas chromatographic/mass spectrometric (GC/MS) analysis of the saturated-hydrocarbon fractions These results also revealed that 'normal' steranes (those with methyl groups substituted at the C_{10}- and C_{13} positions) have been completely biodegraded in all samples, and only diasteranes (C_5 and C_{14} methyl substituted) can be identified at low abundance in $m/z = 217$ mass chromatograms The 'normal' steranes are the most susceptible to degradation among tetra- and pentacyclic hydrocarbon biomarkers and their removal indicates that the extent of biodegradative weathering has progressed to at least level 7 on Volkman *et al*'s scale[4], which they describe as "very extensive" alteration

By contrast to the steranes, hopanes in these samples have been altered to varying degrees Figure 1 shows $m/z = 191$ mass chromatograms corresponding to the four samples in Table 1 The distribution in sample 87-2408 (Fig 1*a*), typical of Monterey-derived oils, exhibits several characteristic features such as the occurrence of 28, 30-bisnorhopanes (labelled 28H in Fig 1) and the C_{35} extended hopanes (labelled 35H, Fig 1) in higher abundance than the C_{34} homologues[13 14] These features have been variously related to source rocks deposited in either a sulphur-rich environment, in the case of the bisnorhopanes[14 15], or hypersaline environments, in the case of the C_{35} extended hopanes[16–18] We characterize the distribution in this sample as 'unaltered', although we are not certain how closely it resembles the pristine distribution before any biodegradation Comparison to triterpane distributions of undegraded Monterey-derived oils[13 14] suggests that the hopanes in this sample have not undergone significant alteration

Figure 1*b*, *c* show the effects of increasing biodegradation on

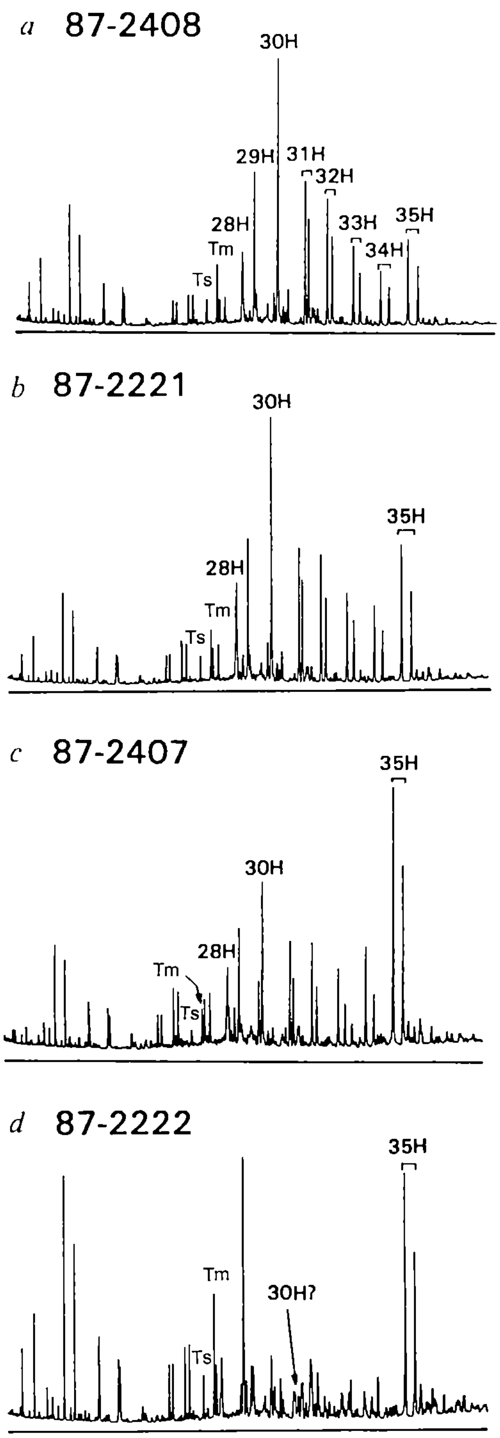

FIG 1 Mass chromatograms of $m/z = 191$ depicting triterpane distributions in Pt Arena tar-sand samples Homologous series of hopanes possessing 17α(H), 21β(H)-stereochemistry are denoted by their carbon number followed by the letter H The two commonly occurring trisnorhopanes are designated Ts and Tm Note the gradual loss of most hopanes relative to the C_{35} compounds with increasing degree of alteration (proceeding from *a* to *d*) In the most severely altered sample (Fig 1*d*) the principal triterpenoid compounds consist of tricyclic terpanes (not labelled), the trisnorhopanes Ts and Tm, the C_{35} hopanes, and various unidentified components Bisnorhopane was not detected in this sample Calculated concentrations of several hopanes (Table 1) indicate that the C_{35} compounds are being selectively preserved Chromatographic conditions 25-m fused-silica capillary column containing 5% phenyl methyl silicone (0 20-mm inner diameter, 0 33-μm film thickness) temperature programmed from 60 to 220° C at 25 °C min^{-1} and 220 to 325 °C at 2 °C min^{-1} after a 1 0-min isothermal hold, helium carrier gas (1 0 $cm^3 min^{-1}$), injector temperature 275 °C, mass spectrometric analysis carried out in SIM (selected ion monitoring) mode using a Hewlett-Packard 5970 Mass Selective Detector

TABLE 1 Composition of extractable organic matter and calculated values for various hopane parameters in Pt Arena tar sands

Sample no	Content (p p m dry weight)	Sulphur (weight %)	SATS	Composition* AROS (relative %)	Composition* HETS (relative %)	ASPH	^{13}C† SATS	^{13}C† AROS	Hopane ratios‡ 28/30	Hopane ratios‡ 35/30	Hopane ratios‡ %22*S*	Hopane ratios‡ Ts/Tm	Concentrations§ 35H	Concentrations§ 30H	Concentrations§ 28H
87-2408	30,000	3 2	11 1	45 2	26 2	16 5	−23 3	−22 7	0 55	0 90	57	0 45	438	485	265
87-2221	72,000	4 2	10 3	42 2	26 2	19 9	−23 3	−22 7	0 82	1 59	58	0 50	452	284	233
87-2407	126,000	5 8	3 7	20 1	21 6	54 1	−23 5	−22 7	0 94	4 99	59	0 50	369	74	70
87-2222	76,000	4 5	9 2	47 2	20 2	22 2	−23 3	−22 8	NC	14 49	62	0 37	461	32	NC

NC=Not calculated

* SATS, AROS, HETS, ASPH=saturated and aromatic hydrocarbons, heterocompounds and asphaltenes, respectively

† ^{13}C composition reported as $\delta^{13}C=[(^{13}C/^{12}C)_{sample}/(^{13}C/^{12}C)_{standard}]-1$

‡ 28/30=28,30-bisnorhopanes/17α(H), 21β(H)-hopane, 35/30=C$_{35}$ extended hopanes (22*S*+22*R*)/17α(H), 21β(H)-hopane, %22*S*=C$_{31}$ extende hopanes (22*S*)/(22*S*+22*R*), T/T=17α(H)-trisnorneohopane/18α(H)-trisnorhopane

§ 28H, 30H, 35H=Concentrations of 28, 30-bisnorhopanes, 17α(H), 21β(H)-hopane and C$_{35}$ extended hopanes (22*S*+22*R*), respectively, expressed pe unit pentane-soluble extractable material Concentrations were calculated relative to an added internal standard (5α(H)-androstane) and are corrected fc differences in gas chromatograph/mass spectrometer response

the hopanes These include a preferential decrease in the abundance of 17α(H), 21β(H)-hopane (labelled 30H, Fig 1) relative to other hopanes and a slight decrease in the abundance of the 22*R* isomer of the C$_{33}$ and C$_{34}$ extended hopanes relative to the 22*S* isomer Most striking, however, is the increasing abundance of the 22*S* and 22*R* isomers of the C$_{35}$ hopanes relative to other hopanes This effect can be seen at its incipient stages in sample 87-2407 (Fig 1c) In the most severely altered sample (sample 87-2222), hopanes containing between 28 and 34 carbons have either disappeared or are present in exceedingly low abundance, and the C$_{35}$ compounds are prevalent (Fig 1*d*) The two most commonly occurring trisnorhopanes, designated Ts and Tm[9], also seem to remain relatively unaltered throughout the entire biodegradation sequence

The trends shown in Fig 1 are quantified in Table 1 The calculated bisnorhopane/hopane ratio shows an increase (from 0 55 to 0 94), suggesting that hopane is degraded slightly more rapidly during the incipient stages of biodegradation Similarly, the 22*S*% values (calculated from C$_{31}$ extended hopanes) increase very slightly with increasing extent of alteration, although we consider the value calculated for sample 87-2222 uncertain because of the difficulty in unambiguously identifying these compounds at the advanced stages of hopane alteration evident in this sample By comparison, the ratio of C$_{35}$/C$_{30}$ hopanes increases progressively from 0 90 to 14 49 with increasing extent of biodegradative alteration (Table 1) The Ts/Tm ratio changes little in the four samples

In the most degraded samples we can document the formation of 25-normethyl homologues Figure 2 shows mass chromatograms of $m/z=177$ and 191 for a severely altered sample, 87-947 The $m/z=177$ ion is diagnostic for triterpanes demethylated in the A/B ring system[4] Overall, the distribution depicted in the $m/z=177$ chromatogram is similar to the 'unaltered' hopane distribution (Fig 1*a*), but differs in several respects We attribute some of these differences to the fact that the response at $m/z=177$ for 25-normethyl hopanes is not the same as that for the corresponding hopane at $m/z=191$ Thus the greater abundance of the demethylated C$_{28}$ compound in the $m/z=177$ chromatogram relative to the C$_{29}$ hopane in the $m/z=191$ chromatogram is due to differences in the mass spectra of the respective compounds Two characteristics of the respective distributions, however, are truly different First, the demethylated C$_{34}$ homologues of the C$_{35}$ hopanes occur at much lower abundance than in the 'unaltered' hopane distribution Second, the demethylated C$_{26}$ homologues of the trisnorhopanes Ts and Tm are absent The latter result differs from studies of biodegraded Australian oils, in which demethylation of these compounds with a distribution similar to that of the hopanes was observed[4] Both observations indicate selective preservation of these compounds in severely altered samples after most other hopanes have been degraded (such as in Fig 1*d*) These feature are illustrated in Fig 2

Several explanations can be invoked to account for the preservation of the C$_{35}$ extended hopanes The carbon skeleton o these compounds very closely resembles that of the bacteriohopanetetrol precursor Because bacteria mediate th degradation process, it might be suggested that these compound were added to the bituminous material in the tar sands durin the biodegradation process through the incorporation of microbial remains We can dismiss this argument for several reasons Absolute biomarker concentrations, calculated relative to a added internal standard, indicate that the concentration of th C$_{35}$ compounds is relatively invariant from the least to the mos severely degraded sample Whereas concentrations of bisnorhopane and hopane shown in Table 1 decrease with increasin degree of alteration, concentrations of C$_{35}$ extended hopane remain relatively constant at 369-461 p p m The lowest C$_{35}$-hopane concentration is associated with the one sample having a significantly higher asphaltene content (Table 1), which migh account for the slightly diminished value This constancy would

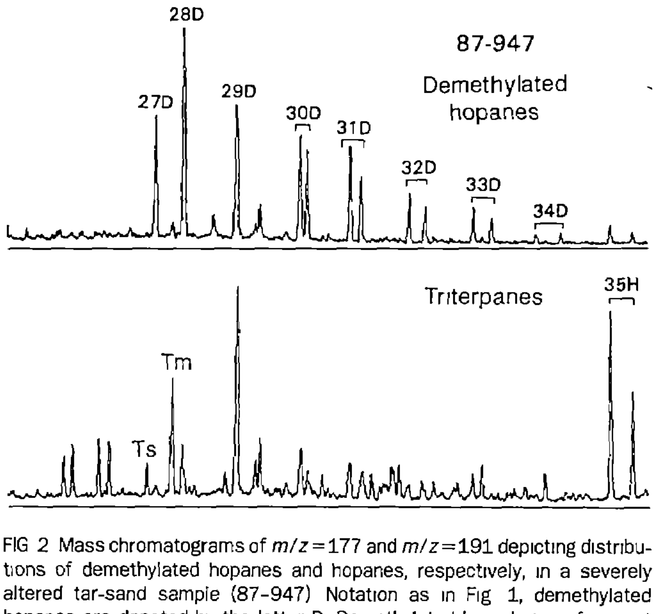

FIG 2 Mass chromatograms of $m/z=177$ and $m/z=191$ depicting distributions of demethylated hopanes and hopanes, respectively, in a severely altered tar-sand sample (87-947) Notation as in Fig 1, demethylated hopanes are denoted by the letter D Demethylated homologues for most hopanes shown in Fig 1*a* are present in the $m/z=177$ chromatogram with the exception of the trisnorhopanes Ts and Tm and the C$_{35}$ hopanes These compounds are the only hopanes recognized in the $m/z=191$ chromatogram Chromatographic conditions as in Fig 1, mass spectrometric analysis conducted using a Finnigan 8222 magnetic/electric-sector instrument (1 000 resolution)

be more characteristic of selective preservation, as it would be very unusual for these compounds to be added at the same rate as they are removed, and also rules out mechanisms involving progressive degradation of the C_{35} compounds at a greatly diminished rate relative to the other hopanes. Further, it would be highly unusual for these compounds to come from a biological source in the geologically preferred distribution, nearly completely isomerized at the C_{22} position (Fig. 1*d*).

The most plausible explanation for these observations is that, under the surface weathering conditions to which these tar sands have been exposed, the C_{35} extended hopanes are resistant to biodegradation. The same conclusion would seem to apply for the trisnorhopanes Ts and Tm. To the best of our knowledge, this hopane biodegradation sequence has not been reported previously. It is therefore difficult to assess the extent of its occurrence in the alteration of triterpanes in biodegraded oils. Enhanced C_{35}-hopane abundances are characteristic of specific environments of deposition, hence these observations may only apply to biodegradation of oils generated from source rocks such as carbonates, hypersaline sources or Monterey Formation analogues. Our observations are, however, somewhat serendipitous because they came about as a result of analysis of various tar-sand samples from the same location. Similar degradation sequences might go undetected unless several samples of the same oil that were biodegraded to varying extents were analysed.

These findings, together with previous studies of crude-oil biodegradation, imply that biodegradative alteration of hopanes can proceed through numerous pathways, which can result in widely differing distributions. Collectively, these pathways are probably more complex than the generalized degradation sequences described by either Volkman *et al.*[4] or Connan[5]. The environmental conditions under which biodegradation takes place (such as the temperature, oxygen level or salinity associated with in-reservoir conditions) may govern the composition of microbial communities active in biodegradative processes[7], which in turn dictate the specific molecular transformations that are observed. This has been substantiated by results of various laboratory simulation studies[8,19]. Caution must be used when evaluating source-rock deposition environments from analyses of hopane distributions in oils[16,18], particularly if the assessments involve analysis of petroleum seepage[20], as the alteration effects associated with biodegradation can be severe. ☐

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1. Ourisson, G., Albrecht, P. & Rohmer, M. *Pure appl. Sci.* **10,** 709–729 (1979).
2. Rullkotter, J. & Philp, P. *Nature* **292,** 616–618 (1982).
3. Seifert, W. K. & Moldowan, J. M. *Geochim. cosmochim. Acta* **43,** 111–126 (1979).
4. Volkman, J. K., Alexander, R., Kagi, R. I. & Woodhouse, G. W. *Geochim. cosmochim. Acta* **47,** 785–794 (1983).
5. Connan, J. in *Advances in Petroleum Geochemistry* Vol. 1 (eds Brooks, J. & Welte, D.) 299–335 (Academic, London, 1984).
6. Hoffman, C. F. & Strausz, O. P. *Am. Ass. Petrol. Geol.* **70,** 1113–1128, 1986.
7. Seifert, W. K., Moldowan, J. M. & Demaison, D. J. *Org. Geochem.* **6,** 633–643 (1984).
8. Goodwin, N. S., Park, P. J. D. & Rawlinson, T. in *Advances in Organic Geochemistry 1981* (eds Bjoroy, M. *et al.*) 650–658 (Wiley, Chichester, 1983).
9. Seifert, W. K. & Moldowan, J. M. *Geochim. cosmochim. Acta* **42,** 77–95 (1978).
10. Orr, W. I. *Org. Geochem.* **10,** 499–516 (1986).
11. Magoon, L. & Isaacs, C. M. in *Petroleum Generation and Occurrence in the Miocene Monterey Formation, California* (ed. Isaacs, C. M. *et al.*) 201–211 (Soc. Econ. Paleontologists Mineralogists, 1983).
12. Bailey, N. J. M., Jobson, A. M. & Rogers, M. A. *Chem. Geol.* **11,** 203–211 (1973).
13. Sofer, Z. *Org. Geochem.* **13,** 939–945 (1988).
14. Curiale, J. A., Cameron, D. & Davis, D. V. *Geochim. cosmochim. Acta* **49,** 271–288 (1985).
15. Grantham, P. J., Posthuma, J. & de Groot, K. in *Advances in Organic Geochemistry 1979* (eds Douglas, A. G. & Maxwell, J. R.) 29–38 (Pergamon, Oxford, 1980).
16. Connan, J. & Dessort, D. *Org. Geochem.* **11,** 103–113 (1987).
17. ten Haven, H. L., de Leeuw, J. W. & Schenck, P. A. *Geochim. cosmochim. Acta* **49,** 2181–2191 (1985).
18. ten Haven, H. L. *et al.* in *Lacustrine Petroleum Source Rocks, Geol. Soc. Spec. Publ. 40* (eds Fleet, A. J. *et al.*) 123–140 (Blackwell Scientific, Oxford, 1988).
19. Rubinstein, I., Strausz, O. P., Spyckerelle, C., Crawford, R. J. & Westlake, D. W. S. *Geochim. cosmochim. Acta* **41,** 1341–1353 (1977).
20. Kennicutt, M. C. & Brooks, J. M. *Appl. Geochem.* **3,** 573–582 (1988).

Bacterial mats from Crater Lake, Oregon and their relationship to possible deep-lake hydrothermal venting

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CRATER Lake is located in a caldera on Mt Mazama, a volcanic centre in the Oregon Cascades which has been active for more than 400,000 years[1]. The 594-m-deep lake is a consequence of a climactic eruption which occurred 6,845 ± 50 years ago; however, caldera volcanism took place as recently as 4,000 years ago[1]. It has been suggested that some of the physical and chemical features of the lake result from hydrothermal inputs[2–7]. Here we present submersible observations of the bottom of Crater Lake, which reveal communities of bacteria that are usually associated with anomalously warm, saline waters. The bacteria seem to derive energy from the oxidation of ferrous iron to fuel their metabolism. We propose that the mats are indicators of diffuse hydrothermal venting into the deep lake.

During August of 1988, 16 dives in the one-person submersible *Deep Rover* were carried out in the lake. Nine dives were in the south-central part of the lake, an area of high heat flow[4] and anomalous compositions and temperatures of the bottom water[7]. We observed bacterial mats at six locations in this area; at some sites multiple mats were present. All mats observed were within 300 m of each other and were either on or near an escarpment with a north-east/south-west orientation. The mats are gelatinous, low-density, 15–300 cm in diameter and 2–15 cm thick. Some of the largest ones are draped over steep rock outcrops (Fig. 1), and the smaller mats lie directly on sediments. Sediments near the mats are dark and contrast sharply with the normal buff-coloured sediments. The maximum water temperature anomaly in the immediate vicinity of the mats was 0.1 °C. Insertion of a temperature probe into the mat, however, revealed temperatures ranging from 4.70 to 9.52 °C, up to 6.0 ° above the typical 3.5 °C bottom water. In addition to the

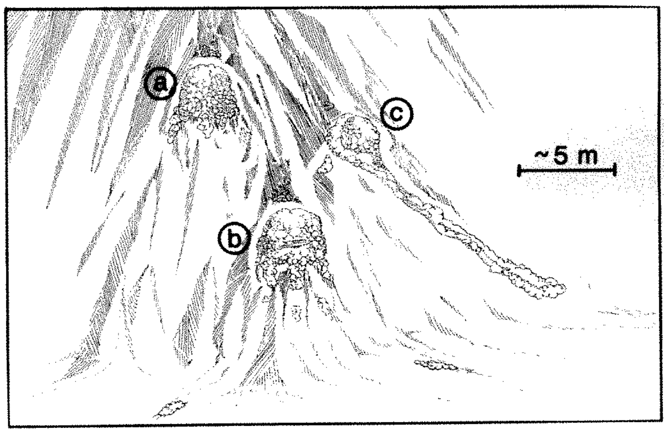

FIG. 1 A schematic drawing of bacterial mats observed during dive 179. The three principal mats observed at this site are identified by 'a', 'b', 'c'. These large mats seem to be attached to rocky cliff faces. Mat 'c' extends down the cliff face a further 10 m below the primary structure. This extension seems to be actively growing and not simply a consequence of pieces that roll down the slope. Small mats are growing at the base of the slope on sediments. The sediments along the base of the slope are discoloured, apparently because of Fe and Mn precipitation. Mat sample and mat waters shown in Tables 1 and 2 were recovered from 'a'.

TABLE 1 The composition of the bacterial mat and other iron-rich deposits

	Bacterial mat	Galapagos mounds[26]	Santorini[27]	Drive 187 crust*
Al	0.21	0.32	0.87	0.46
Si	7.2	13.3	6.8	11.7
P	3.7	—	0.11	1.96
Ca	0.8	1.4	1.3	0.87
Fe	37.9	27.5	27.8	35.5
Mn	0.014	7.9	0.045	0.45
As	0.36	—	—	0.08
Ba	0.02	0.07	0.005	0.05
S	0.3	—	0.41	—
C	2.44	—	4.0	—
N	0.97	—	—	—

All values are in weight per cent as determined by complete dissolution with HF/HNO_3 acids and analysis by atomic absorption spectrophotometry. The dashes indicate that no measurements were made.

* A lithified crust recovered from a sedimented bottom in the general region of the bacterial mats.

temperature anomalies associated with the mats, waters with anomalous concentrations of ^3He, Mn, ^{222}Rn, Li, Si and Cl have been observed at and near these features[8-10].

A predominantly bacterial composition for the mats (genera *Gallionella* and *Leptothrix*) is confirmed by scanning electron microscopy (Fig. 2). The sheath-forming *Gallionella* is generally considered to be a chemolithoautotroph[11] as it requires a source of reduced iron[12] and will grow in the absence of significant organic carbon[13]. It is not necessarily thermophilic because it has been found in low-temperature springs, wells and drainages[12], but this genus has been observed in thermal springs with temperatures up to 47 °C (ref. 14). Because of the accumulations of ferric hydroxide and manganese dioxide accumulations on the sheaths of *Leptothrix*, these bacteria are thought to oxidize ferrous and manganous ions[15]. An autotrophic metabolism for *Leptothrix* has not been demonstrated, but, like *Gallionella*, it seems to thrive in environments that provide a gradient between reduced and oxidized forms of iron and manganese. *Leptothrix* is also not a thermophilic form, but it has been observed in the recently discovered thermal springs on Loihi Seamount, the most eastward extension of Hawaiian-chain volcanism[16]. There thick mats similar to the Crater Lake communities surround vents with temperatures that range between 15 and 30 °C (ref. 17).

In Table 1, the bulk composition of a bacterial mat is compared with that of hydrothermal iron oxide precipitates from several marine hydrothermal deposits and some iron-rich crusts collected in the vicinity of the Crater Lake bacterial mats. The mat contains >35% iron, which accounts for >70% of the mass of the mat (as FeOOH). Significant amounts of silica and phosphorus are also present. The relatively low sulphur content indicates that sulphur-oxidizing bacteria, which would transform hydrogen sulphide into elemental sulphur or sulphate, are not important members of the bacterial community. The concentrations of iron, aluminium and silicon in the mats and the hydrothermal oxide deposits are similar. The composition of the lithified crust is very similar to that of the bacterial mat.

Although the scanning electron micrographs demonstrate that the bacterial mats are predominantly composed of sheath-forming bacteria, only 2.4% of the material is organic carbon. It is probable that most of the bacteria shown in Fig. 2 are remnant sheaths rather than viable bacteria. Analyses of the organic fraction demonstrate that the extractable lipids are simple fatty acids. Sterols and other indicators of phytoplankton, zooplankton or local terrigenous sources are absent. In addition, the atomic ratio of carbon to nitrogen in the mat (2.9) is low compared with organic matter from plankton and is similar to bacterial sources of organic matter[18]. These analyses are consistent with fresh organic matter of microbial origin.

Within the general area where bacterial mats were found, we also observed lithified crusts of iron- and manganese-enriched material similar to the crust reported in Table 1. At some locations these deposits formed continuous pavements several metres across. At other sites multicoloured, pebble-sized material overlay what seemed to be normal sediments. The pebbles seem to be fragments of the lithified crusts.

Water associated with a bacterial mat was sampled by placing the open end of a cylindrical water sampler directly within the

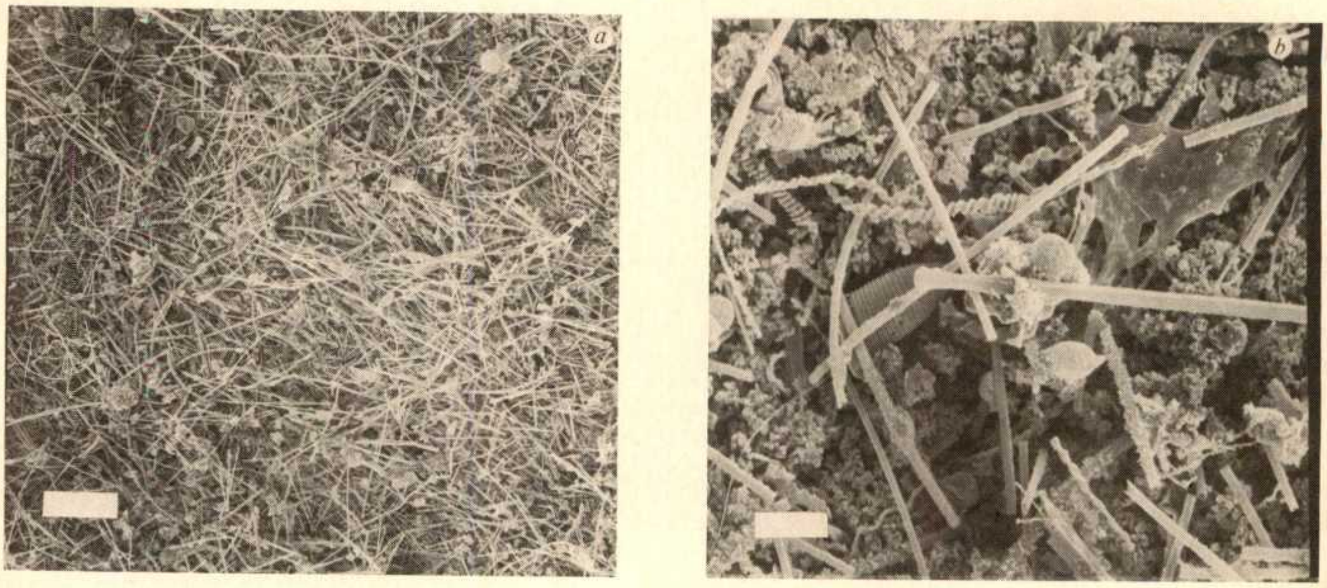

FIG. 2 *a*, A low-power scanning electron micrograph of the bacterial mat from dive 179. Tentative identification indicates these forms are iron-oxidizing sheath-forming bacteria of the genera, *Gallionella* and *Leptothrix*. The scale bar is 100 μm in length. *b*, A scanning electron micrograph of the bacterial mat from dive 179. The scale bar is 10 μm in length. The genus, *Gallionella* are the forms with twisted sheaths; *Leptothrix* are the straight sheaths. The ribbed dome-like form in the centre is thought to be a protozoa. This form is seen on many of the scanning electron micrographs and could be feeding on the bacteria. The tube-like form in the centre-right with a blunt end is part of a diatom.

TABLE 2 Chemical composition of bacterial mat water and normal deep lake water

Sample	Na ($\mathrm{mg\,l^{-1}}$)	K ($\mathrm{mg\,l^{-1}}$)	Ca ($\mathrm{mg\,l^{-1}}$)	SO_4 ($\mathrm{mg\,l^{-1}}$)	Cl ($\mathrm{mg\,l^{-1}}$)	SiO_2 ($\mathrm{mg\,l^{-1}}$)	Li ($\mathrm{\mu g\,l^{-1}}$)	Mn (nM)
7.3 (hydrocast, 1987, 351 m)	10.4	1.71	6.73	10.2	10.6	17.3	44.1	0.6
CD179BT (bacterial mat water)	19.3	3.01	12.6	20.4	15.7	28.7	77.2	590

All analyses are by atomic absorption spectrophotometry.

mat (~10% of the sampler body penetrated the mat). After closing, the sampler retained several centimetres of mat material and a water sample that represents a mixture of the 'mat water' and of the local bottom water around the mat.

The bacterial-mat water sample contains the highest concentration of dissolved ions of any water sample collected within the caldera (Table 2). The concentrations of the major ions are approximately double the lake-water values, and the Mn concentrations are one thousand times higher than deep lake water. The mat water is an appropriate chemical 'endmember' that could explain the anomalously high concentrations of ions in Crater Lake[10]. Because of the sampling method, the extent of dilution of the bacterial-mat fluids by normal lake water is uncertain. A conservative estimate, based on relative volumes alone, suggests a 1:10 dilution of mat water by lake water. Thus, *in situ* mat-water concentrations are probably an order of magnitude larger than those shown in Table 2.

Elevated temperatures of fluids within the mat indicate geothermal heating. The contribution of heat by bacterial metabolic activity can be shown to be insignificant. (Assuming that the heat produced by oxidation of all dissolved ferrous ions in mat fluids is retained within the mat an upper limit to the metabolic heat production can be calculated. Using this closed-system assumption, the increase in temperature is a function of the concentration of iron in the fluids. An appropriate reaction is:

$$Fe^{2+} + \tfrac{3}{2}H_2O + \tfrac{1}{4}O_2 \rightleftharpoons FeOOH(s) + 2H^+$$

The enthalpy of this reaction[19] is $-9.2\ \mathrm{kcal\,mol^{-1}}$. Assuming an upper limit of 1.0 mM iron[20], conversion of all the dissolved iron to solid form would add ~9 cal of heat to each litre of water. Thus, the reaction would raise the temperature of that water by less than 0.01°.) Chemical geothermometers[21,22] permit an estimate of the water–rock reaction temperature of mat fluids. Assuming the mat-water sample (Table 2) was diluted ten times with normal lake water, the original concentration would be: $Na = 99\ \mathrm{mg\,l^{-1}}$; $Ca = 65\ \mathrm{mg\,l^{-1}}$; $Mg = 37\ \mathrm{mg\,l^{-1}}$; $K = 15\ \mathrm{mg\,l^{-1}}$; $SiO_2 = 128\ \mathrm{mg\,l^{-1}}$. If chalcedony (a microcrystalline form of quartz) is the phase controlling the silica content of the fluids, we estimate a temperature of 81 °C for the rock equilibration temperature of the mat fluid. Using the Na-K-Ca geothermometer[21,22] (with no Mg correction) a temperature of 86 °C is calculated. There may be significant errors in the estimated temperatures because the assumed solubility and exchange reactions are slow to reach equilibrium at these relatively low temperatures and our endmember fluid composition is not well constrained. The result, however, is consistent with a geothermal source of the mat waters, and ~80 °C is our best estimate for the rock equilibration temperature of the geothermal fluids.

We conclude that the mats are produced by bacterial communities that oxidize ferrous iron carried in the fluids advecting through the mat. We base our assertion of fluid advection on the thermal and chemical gradients across the mats and the likelihood that the abundant growth of organisms requires a steady supply of reduced iron. We did not, however, measure or see evidence of flow through the mat, but dispersed and subtle flow of fluids would not be observable in the presence of the physical disturbance around the submarine.

We hypothesize that bacterial mats, lithified crusts and pebbled surfaces represent sequential stages in the evolution of precipitates that form from heated fluids that advect through sediments and rocky outcrops at certain locations on the lake floor. The elevated temperatures and enrichment of these waters in elements commonly associated with thermal waters such as silicon, chlorine and lithium (Table 2) indicates the fluids are the result of hydrothermal circulation. Moreover, ^3He, a definitive tracer of a mantle source, is strongly enriched in lake bottom waters near the bacterial mat[9], compatible with hydrothermal input.

The following scenario can account for the presence of mats and crusts: iron-oxidizing, chemolithotropic bacteria precipitate large amounts of iron in mats at interfaces between the anoxic advecting fluids and oxic lake waters. Because these organisms require a source of ferrous iron, they thrive only as long as the advection of fluids continues. If the thermal-fluid advection is through sediments, as opposed to rock, excess pore pressure and doming of the sediments will result, similar to that observed in sediment-hosted hydrothermal systems in the oceans[23-25]. Precipitation within the conduits of hydrothermal systems, however, eventually blocks the flow and venting stops at a given site. When this occurs, the bacterial mat is transformed from a gelatinous living structure to a lithified crust. Cessation of venting would result in dome collapse and breaking of the lithified crusts would account for the pebble fields of mixed crust varieties. □

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1. Bacon, C. & Lanphere, M. A. in *Crater Lake: An Ecosystem Study* (eds Drake, E. T., Larson, G. L., Dymond, J. & Collier, R.) (Am. Ass. Adv. Sci., San Francisco, in the press).
2. Van Denburgh, A. S. *Geol. Surv. prof. Pap. U.S.* No. 1005, 58–60 (1968).
3. Simpson, H. J. thesis, Columbia Univ. (1970).
4. Williams, D. L. & Von Herzen, R. P. *J. geophys. Res.* **88,** 1094–1104 (1983).
5. Thompson, J. M., Nathenson, M. & White, L. D. in *Crater Lake: An Ecosystem Study* (eds Drake, E. T., Larson, G. L., Dymond, J. & Collier, R.) (Am. Ass. Adv. Sci., San Francisco, in the press).
6. Nathenson, M. in *Crater Lake: An Ecosystem Study* (eds Drake, E. T., Larson, G. L., Dymond, J. & Collier, R.) (Am. Ass. Adv. Sci., San Francisco, in the press).
7. Collier, R. W., Dymond, J., McManus, J. & Lupton, J. in *Crater Lake: An Ecosystem Study* (eds Drake, E. T., Larson, G. L., Dymond, J. & Collier, R.) (Am. Ass. Adv. Sci., San Francisco, in the press).
8. Collier, R. *et al. Eos* **68,** 1721 (1987).
9. Lupton, J. E., Collier, R. & Dymond, J. *Eos* **68,** 1722 (1987).
10. Collier, R. W. & Dymond, J. *Studies of Hydrothermal Processes in Crater Lake* Rep. No. 79 CPSU (Oregon State Univ., Corvallis, 1989).
11. Ehrlich, H. L. *Geomicrobiology* 393 (Dekker, New York, 1981).
12. Hanert, H. H. in *The Prokaryotes: A Handbook on Habitats, Isolation, and Identification of Bacteria* (eds Starr, M. P., Stolp, H., Truper, H. G., Balows, A. & Schlegel, H. G.) 509–515 (Springer, Berlin, 1981).
13. Wolfe, R. S. in *Principles and Applications in Aquatic Microbiology* (eds Heukelakian, H. & Dondero, N. C.) 82–97 (Wiley, New York, 1964).
14. Hanert, H. H. *Geol. Rdsch.* **62,** 786–812 (1973).
15. Mulder, E. G. & Deinema, M. H. in *The Prokaryotes: A Handbook on Habitats, Isolation, and Identification of Bacteria* (eds Starr, M. P., Stolp, H., Truper, H. G., Balows, A. & Schlegel, H. G.) 425–440 (Springer, Berlin, 1981).
16. Karl, D. M., Brittain, A. & Tilbrook, B. *Global Venting, Midwater and Benthic Ecological Processes* (eds DeLuca, M. P. & Babb, I.) (U.S. Dept of Commerce, Washington, DC, 1988).
17. Karl, D. M., McMurtry, G. M., Malahoff, A. & Garcia, M. O. *Nature* **335,** 532–535 (1988).
18. Goldman, J. C., Caron, D. A. & Dennett, M. R. *Limnol. Oceanogr.* **32,** 1239–1252 (1987).
19. Garrels, R. M. & MacKenzie, F. T. in *Equilibrium Concepts in Natural Water Systems* (*151st Meet. Am. chem. Soc.*) 222–242 (Am. chem. Soc., Washington, DC, 1967).
20. Mariner, R. H., Presser, T. S. & Evans, W. C. *Geol. Surv. Open-File Rep. 82–98* (1982).
21. Fournier, R. O. in *Geothermal Systems: Principles and Case Histories* (eds Ryback, L. & Muffler, L. P. J.) (Wiley, New York, 1981).
22. Fournier, R. O. & Potter, R. W. *Geochim. cosmochim. Acta* **43,** 1543–1550 (1979).
23. Williams, D. L. *et al. J. geophys. Res.* **84,** 7467–7484 (1979).
24. Maris, C. R. P., Bender, M. L., Froelich, P. N., Barnes, P. N. & Luedtke, N. A. *Geochim. cosmochim. Acta* **48,** 2331–2346 (1984).
25. Wheat, C. G. & McDuff, R. E. *Eos* **69,** 1499 (1988).
26. Corliss, J. B., Lyle, M., Dymond, J. & Crane, K. *Earth planet. Sci. Lett.* **40,** 12–24 (1978).
27. Bostrom, K. & Widenfalk, L. *Chem. Geol.* **42,** 203–218 (1984).

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The earliest known reptile

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AMNIOTES (reptiles, birds and mammals) are distinguished from non-amniote tetrapods (amphibians) by the presence of complex embryonic membranes. One of these, the amnion, gives its name to the group. Very few skeletal characters distinguish amniotes from amphibians[1], making it difficult to recognize early amniotes in the fossil record. The earliest amniote fossil identified so far is *Hylonomus* from the Westphalian (Upper Carboniferous) of Joggins, Nova Scotia[2,3], (~300 Myr). I report here the discovery of a much earlier amniote skeleton from the Brigantian (Lower Carboniferous) of Scotland (~338 Myr)[4], which thus represents the earliest occurrence of amniotes in the fossil record. The specimen was collected from the East Kirkton Limestone, near Bathgate, West Lothian[4-8], and is part of a unique terrestrial fauna that includes eurypterids, myriapods, scorpions and the earliest-known harvestman spider[7,9], together with the earliest known temnospondyls, a group that may include the ancestors of all living amphibians[10]. It will make an important contribution to our knowledge of early amniote morphology and the interrelationships of tetrapods.

The specimen described here (SPW2326) currently forms part of the private collection of Stanley Wood who found it in the black shale member of the East Kirkton succession. It is preserved in part and counterpart and consists of an almost entire articulated skeleton (Fig. 1) which has been split into halves so that little surface detail of the bones is visible. The bedding plane in which the specimen lay is slightly contorted, disrupting the anterior parts of the skeleton. The total length of the skeleton is between 180-200 mm (presacral length ~120 mm).

The skull has been crushed dorsoventrally. Most of the skull roof is preserved, together with the right cheek and mandible which have been displaced laterally (Fig. 2*a*). Little of the palate is visible, but there is evidence that it is concealed beneath the overlying bones of the skull roof. The snout-occiput length is ~18 mm and the snout-quadrate length ~20 mm. The preorbital region shows a pattern of bones common to all early tetrapods, with midline frontals and nasals flanked laterally by pre- and postfrontals and large premaxillae anteriorly. But, unlike other Carboniferous amniotes such as protorothyridids[11] the pre- and postfrontals are in contact, preventing the exposure of the frontal in the orbital margin (Fig. 2*a*). In the post-orbita region the midline parietals appear to contact the postorbita and squamosal. There is no evidence that the temporal series (intertemporal, supratemporal and tabular) intervenes between the parietals and cheek bones. The squamosal, postorbital, juga and quadratojugal are arranged in the primitive tetrapod pattern[12]. Nine sclerotic plates are preserved in the dorsal half o the orbit.

The vertebral column is preserved in articulation, although in places the bone is eroded, making it difficult to distinguish individual bones. There are approximately 32 presacral vertebrae and a similar number make up the preserved portion o the tail. The first eight presacral vertebrae are preserved in dorsa view, the remainder in lateral aspect. The form of the vertebrae is illustrated in Fig. 2*b*. The hourglass-shaped centrum seems to be fused to the neural arch which bears a short neural spine Between each successive centrum is a wedge-shaped intercentrum. This is relatively much larger than those of the Westphalian protorothyridids[2], but much smaller than those of other reptiliomorphs from East Kirkton[6,7]. The neural arches are narrow and unswollen, and all the presacral vertebrae seem to bear ribs.

The pectoral girdle and fore limbs are incompletely preserved, and will require detailed study before they can be adequately described, but the pelvic girdle and hind limbs are very well preserved. They lie below the vertebral column except that the left pes overlies vertebrae in the sacral region, obscuring detail of the pattern of its bones. The right pelvis and hind limbs are illustrated in Fig. 2*c*. The camera lucida drawing was made of the bones as preserved on the counterpart block and thus appears to be from the left side of the skeleton. The dorsoventrally flattened pelvis consists of a large puboischiadic plate 16 mm long and a relatively short, narrow iliac blade ~6 mm long. The limb bones are long and gracile. The femur is 11 mm long and the tibia and fibula are each ~6 mm long. The right pes is very well ossified and the majority of bones are preserved. Two large proximal tarsal bones are readily identified and are almost certainly the homologues of the astragalus and calcaneum of extant amniotes. Most of the centralia are preserved in close apposition to the metatarsals, but the distal bones of the foot have folded back towards the epipodials. The digits are very

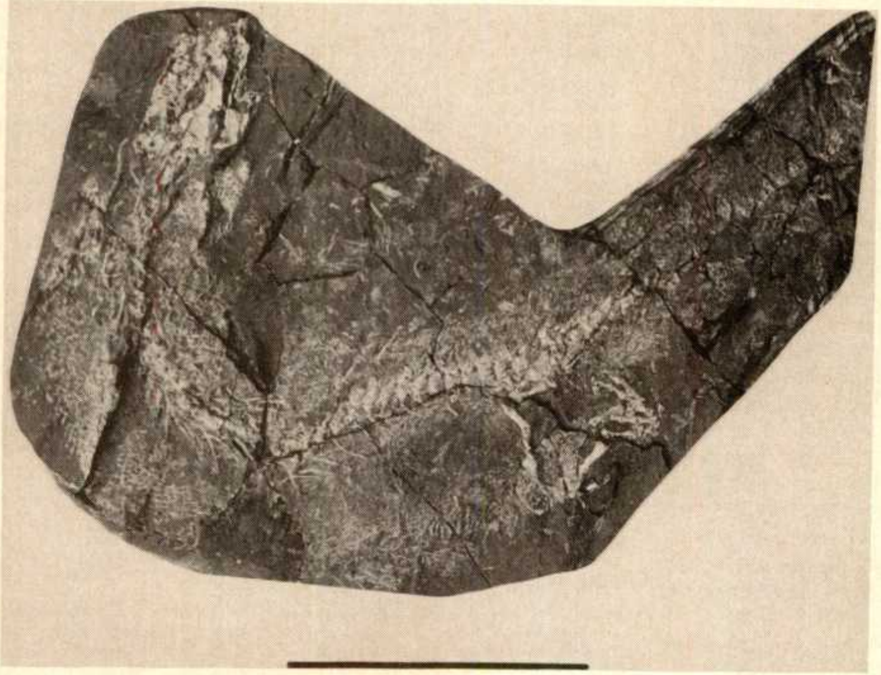

FIG. 1 Fossil amniote skeleton from the East Kirkton Limestone (SPW2326). Counterpart block. Scale bar, 50 mm.

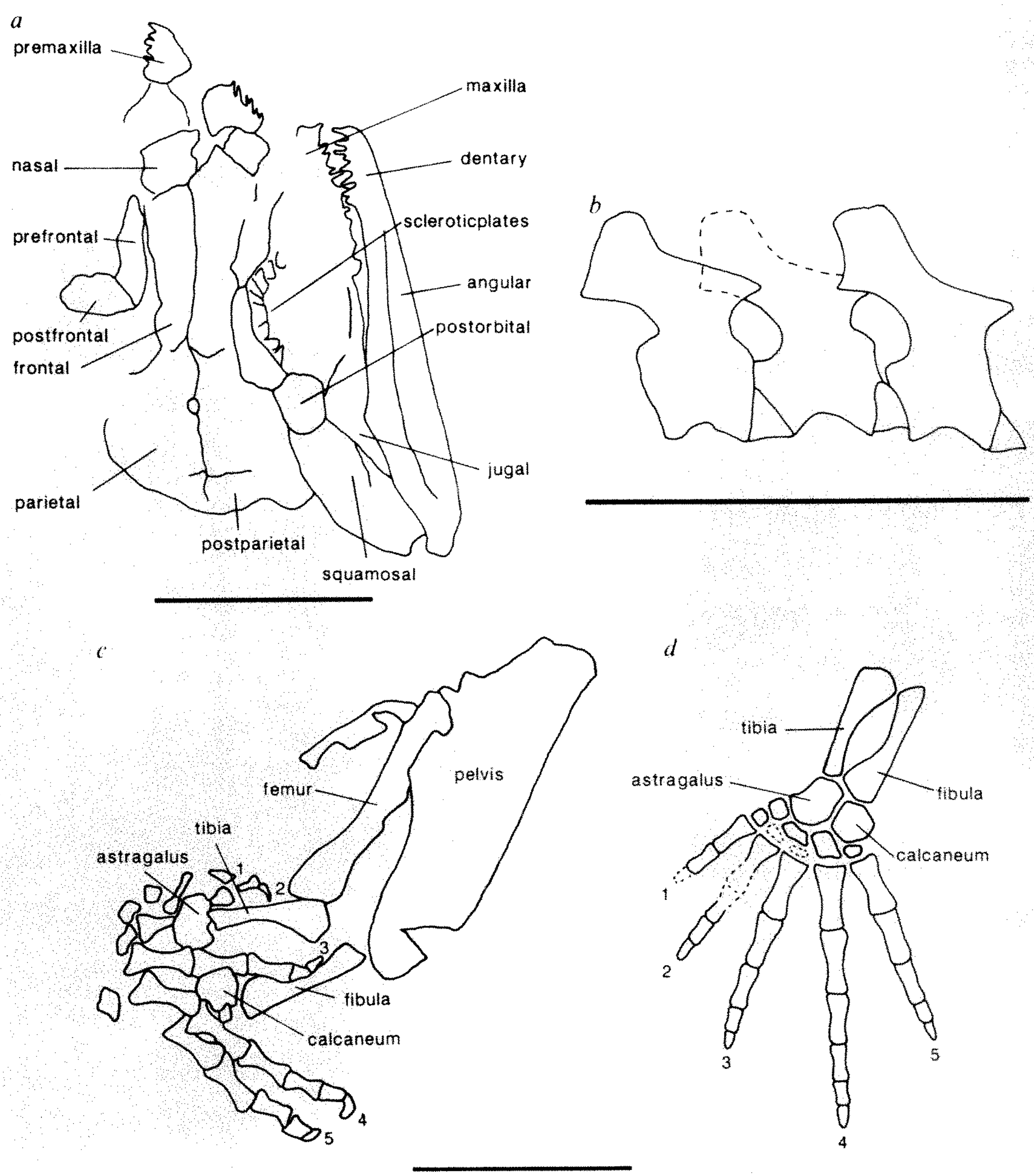

FIG. 2 Fossil amniote (SPW2326). *a*, Skull, dorsal view. Composite figure based on data from part and counterpart halves. *b*, Trunk vertebrae (approximate presacral numbers 20–22), lateral view. *c*, Right pelvis and hind limb drawn from counterpart block. *d*, Restoration of right tibia, fibula and pes, reversed. Scale bars, 10 mm.

well-articulated and the pattern of phalanges is clear in all but the first digit, in which the terminal phalanx appears to be missing. The phalangeal formula is 23454. A restoration of the tibia, fibula and pes is shown in Fig. 2*d*.

SPW2326 is placed in the Amniota on the basis of the presence of an astragalus and calcaneum (a character it shares with extant amniotes) and the reduction of the temporal series to permit contact between the parietal, the postorbital and the squamosal (a character shared with other Carboniferous amniotes). Among Palaeozoic tetrapods, the presence of an astragalus and calcaneum is not a unique feature of amniotes: these bones have been described in a number of microsaurs[13]. But the microsaur skull is characterized by a single large bone in the temporal series, usually described as the tabular, which separates the midline parietal from the postorbital and squamosal bones of the cheek. In addition, no microsaur has been described with a pedal phalangeal formula 23454, although this pattern has been restored in *Tuditanus*[14]. No other features are evident on SPW2326 that would indicate relationship with other groups of Carboniferous amphibians. The pattern of the skull roofing bones of SPW2326 precludes relationship with one of the groups of non-amniote reptiliomorphs (see ref. 1) or with the taxon including ichthyostegids, colosteids and temnospondyls. It lacks the unique vertebral morphology of nectrideans[15] and the aberrent cranial morphology of lysorophids and adelogyrinids[13], whereas the presence of limbs excludes it from the Aïstopoda.

SPW2326 is thus considered to be an amniote. However, its position within the Amniota remains to be determined and must await detailed description. The absence of lateral temporal openings in the skull clearly precludes inclusion in the Synapsida or Diapsida, but its relationships with the other two major groups of Carboniferous amniotes, the Captorhinidae and Protorothyrididae[11] are unclear. It remains to be seen whether the discovery of SPW2326 will help to resolve the problem of the relationship of amniotes to non-amniotes. Recent discussion of this problem has been dominated by an analysis of characters from the braincase and otic region of Upper Carboniferous and Lower Permian taxa[1,16,17]. But, as the whole skeleton of the earliest amniote becomes better known, and the other reptiliomorphs from East Kirkton are described, it is anticipated that new characters will be discovered which will provide tests for current phylogenetic hypotheses. ☐

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1. Panchen, A. L. & Smithson, T. R. in *The Phylogeny and Classification of the Tetrapods, Vol. 1: Amphibians, Reptiles, Birds* (ed. Benton, M. J.) 1–32 (Clarendon, Oxford, 1988).
2. Carroll, R. L. *J. Linn. Soc. (Zool.)* **45,** 61–83 (1964).

3. Dawson, J. W. *Air-breathers of the Coal Period* (Dawson Brothers, Montreal, 1863).
4. Wood, S. P., Panchen, A. L. & Smithson, T. R. *Nature* **314,** 355-356 (1985).
5. Smithson, T. R. *Scott. J. Geol.* **21,** 123-142 (1985).
6. Milner, A. R. *et al. Mod. Geol.* **10,** 1-28 (1986).
7. Rolfe, W. D. I. *Earth Sci. Cons.* **25,** 22-28 (1988).
8. Muir, R. O. & Walton, E. K. *Trans. geol. Soc. Glasg.* **22,** 157-168 (1957).
9. Rolfe, W. D. I. *et al.* in *Volcanism and Fossil Biotas* (ed. Lockley, M.) (Geological Society of America, Special Paper 244, in the press).
10. Milner, A. L. in *The Phylogeny and Classification of the Tetrapods, Vol. 1: Amphibians, Reptiles, Birds* (ed. Benton, M. J.) 59-102 (Clarendon, Oxford, 1988).
11. Heaton, M. J. & Reisz, R. R. *Can. J. Earth Sci.* **23,** 402-418 (1986).
12. Panchen, A. L. & Smithson, T. R. *Biol. Rev.* **62,** 341-438 (1987).
13. Carroll, R. L. & Gaskill, P. *Mem. Am. phil. Soc.* **126,** 1-211 (1978).
14. Carroll, R. L. & Baird, D. *Amer. Mus. Novit.* **2337,** 1-50 (1968).
15. Milner, A. C. in *The Terrestrial Environment and the Origin of Land Vertebrates* (ed. Panchen, A. L.) 377-405 (Academic, London, 1980).
16. Heaton, M. J. in *The Terrestrial Environment and the Origin of Land Vertebrates* (ed. Panchen, A. L.) 491-551 (Academic, London, 1980).
17. Smithson, T. R. *Zool. J. Linn. Soc.* **85,** 317-410 (1985).

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Evidence from aphasia for the role of proper names as pure referring expressions

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HERE we describe a patient who, as a result of brain damage, had a dramatic inability to retrieve proper names, and who thus offered the opportunity of observing the distinction made in the brain between proper and common names. Although category specific aphasic disturbances are relatively common[1,2], this patient's anomia for proper names is very rare: only two other cases have been described so far[3,4]. The opposite phenomenon, a selective sparing of proper names, has also been recently observed[5]. It is widely assumed that double dissociations such as this reflect the premorbid organization of the cognitive system: the categories of proper and common nouns, therefore, would be separately represented or, at least, separately accessed in the intact brain. The only other such deficits, including the inability to learn arbitrary links between words and to guess the titles of pieces of music, are believed to be indicative of a problem in dealing with purely referential relations. This, in turn, would indirectly confirm the role of proper names as pure referring expressions.

L.S. was a 41-year-old employee in a hardware store, with eight years of education, who suffered a head trauma after a riding accident in July 1988. At that time a computerized tomography documented a left fronto-temporal lesion; in September 1989 NMR scanning showed a similar picture, emphasizing the involvement of basal structures. Clinical examination revealed no neurological signs, and no psychiatric pathological signs were ever observed. Very mild word-finding difficulties were noted. In September 1988 no pathological signs were detectable in his spontaneous speech or in his language comprehension. His residual deficit consisted almost exclusively of a dramatic inability to retrieve proper names either spontaneously or on formal testing. This deficit affected only oral and written word retrieval, because his auditory and written comprehension of proper names were both intact. On neuropsychological examination with standard test batteries[6,7] he obtained normal scores: in particular his visual recognition (including faces), spatial imagery, short- and long-term memory (with the exceptions reported below) were found to be intact. He accomplished perfectly all standard linguistic tasks. The following is a description of his inability to retrieve proper names and of his associated disorders.

In visual-confrontation naming he was 100% correct with real objects (50 items) and he scored 98 out of 100 with pictorial material. Categories shown in previous studies to be often selectively sensitive to brain damage[1] were also separately tested in a confrontation naming paradigm. A minimum of ten items for each of these categories were used as stimuli: L.S. made no errors. His performance with proper names was completely different. With real persons his knowledge of names was limited to those of most members of his family, which he had relearned during the months before testing. He gave correct information, however, about other real persons ("doctor"), and never experienced recognition problems. When presented with pictures of famous persons his naming performance was 2/25. Nonetheless, he provided correct information and details about these people's identities in every case (for example "prime minister"). In all cases he had no problems in matching spoken-aloud or written names with the pictures in a multiple-choice test. This ability to retrieve geographical names was tested using a blank atlas and pictures of the sites of well-known cities. He could name only 6/15 pictures and 4/15 map sites, but he was able to match them correctly in pairs. He also gave correct information about all of the items. In addition he was tested using the blank atlas on rivers (naming 3/8), countries (2/8) and mountains (2/8). Again, for all items, he provided a vast amount of correct information. We confronted him with several popular pieces of wordless classical music. If he demonstrated that he recognized them by singing along and continuing the melody appropriately when the music stopped, then we asked him to name the piece. He was unable to retrieve the title of any of such 12 pieces, regardless of whether their proper names were very abstract ('5th Symphony') or descriptive ('Moonlight Sonata'). He was also unable to provide the names of the composers.

In naming from definition L.S.'s performance was again very satisfactory provided that proper names were not the target. His score was 75/80, the stimuli being various categories of nouns (including animate beings, inanimate objects and abstract nouns), adjectives, verbs and numbers. By contrast, his performance with proper names was very poor; he scored 5/8 for relatives, 2/15 for famous persons, 3/5 for cities, 1/5 for mountains and 3/5 for countries. He could point correctly to all the geographical locations on the blank atlas. Phonological cueing (consisting of the first phoneme of the name) alone never improved his performance in generating proper names on either confrontation or definition. Semantic cueing was more effective, but only in the particular case in which the proper name also had a real meaning. For instance, Colombo (Columbus) in Italian means 'pigeon': L.S. seemed to be helped if the description included a cue to the meaning of the name ("Tell me who discovered America. He had the name of a bird.") His performance was correct in 4 out of 15 items of this kind, and when helped also with a phonetic cue he was able to score 9/15.

Naming by category in one minute was also tested. In this case he provided the names of 12 fruits, 11 vegetables, 9 birds, 13 clothes, 6 forms of footwear, 16 body parts, 15 means of transportation, 13 occupations, 12 sports, 7 carpenter's tools, 10 types of pasta, 13 common names beginning with the letter 'F', 9 beginning with 'M' and 10 beginning with 'S'. In the same time limit he was able to generate 12 proper names when "any person's name" was requested, but his performance dropped dramatically when more delimited categories were asked (one musician, two sportsmen, two actors, and two politicians). Additional time did not improve his performance with proper name categories, whereas for all other categories he continued to give new correct items.

L.S. was able to repeat immediately any proper name and to retrieve it if allowed to rehearse it silently without interference. Any interference procedure (such as counting backwards), however, caused his performance to drop to zero in 15 seconds.

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The ability to acquire arbitrary associations between pairs of words, which may be considered as conceptually equivalent to naming proper names, was finally tested. The patient was given the paired-associate learning task from the Wechsler Memory Scale and the Bisiach, Cappa and Vallar[7] battery. He quickly learned the 'easy' pairs (in which the two words have a semantic relation to each other, such as 'north'-'south'), but he showed a chance performance over several attempts in retrieving the second member of the pair, given the first, when the relation between the two was totally arbitrary (such as 'clock'-'pear'). By contrast, he had no difficulty in a word-learning task. Specifically his score on a ten-word free-recall task (three trials) was within the normal range[7]. At work he also had great difficulty in learning numerical labels consistently when applied to certain items of hardware.

Our evidence indicates a common denominator underlying L.S.'s performances: the inability to deal (at the retrieval level) with purely referential nondescriptive semantic relations. Indeed, proper names are believed by some philosophers of language[8,9] to be pure referring expressions. According to Frege's[10] distinction, proper names just carry 'reference', that is they denote the individuals or the entities that are called by them, but have no 'sense', that is, they do not describe any property or imply any attribute. They are the opposite of 'descriptions', which have sense and which encompass all common nouns. Meaningless labels, which is what pure referring expressions are, could indeed need an independent system to take care of them; in L.S. this system would be disrupted. Psychologically, this makes more sense than the alternative explanation that L.S.'s inabilities arose because of a difficulty purely in retrieving proper names; in this case one would be left with the problem of specifying what particular quality makes it useful for the brain to implement proper names independently from common ones. The need of an independent system may still be there even if proper names were not meaningless labels but 'shorthand descriptions', as some authors[11] prefer to the pure reference theory. A descriptive value, varying from none (or very little) to presumably a lot, would be in any case an important dimension in the organization of semantic memory. It would be orthogonal with other dimensions such as the one defined by the prevalence of structural or functional attributes, which also has been suggested to determine category effects in aphasia[12].

There are at present only a few highly speculative models of the actual wiring in the nervous system of such organization: those involving modularly distinct subsystems[12,13] or regions of relative specialization within a distributed net[2,5] are the more accredited ones.

Note added in proof. Note also that a substantially similar argument comes from modern linguistics. In Jackendoff's theory of natural language semantics, an important distinction in conceptual structure is the binary feature Type/Token[14]. What one learns and stores in memory can be linked either with Token (if one is remembering an individual) or with Type (if one has learned a category). Proper names appear typically to denote individuals. ☐

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1. Semenza, C. & Denes, G. *Aphasiology* **2,** 405-410 (1988).
2. McCarthy, R. A. & Warrington, E. K. *Nature* **334,** 428-430 (1988).
3. McKenna, P. & Warrington, E. K. *J. Neurol. Neurosurg. Psychiat.* **43,** 781-788 (1978).
4. Semenza, C. & Zettin M. *Cognitive Neuropsychol.* **5,** 711-721 (1988).
5. Warrington, E. K. McCarthy, R. A. *Brain* **110,** 1273-1296 (1987).
6. Goodglass, H. & Kaplan, E. *The Assessment of Aphasia and Related Disorders.* (Lea & Febiger, Philadelphia, 1972).
7. Bisiach, E., Cappa, S. & Vallar G. *Guida all'esame neuropsicologico* (Cortina, Milan, 1983).
8. Mill, J. S. *A System of Logic* (Longman, London, 1843).
9. Kripke, S. *Naming and Necessity* (Blackwell, Oxford, 1980).
10. Frege, G. in *G. Frege, Funktion, Begriff, Bedeuntung* (ed. Patzig, G.) 40-65 (Vandenhoeck und Ruprecht, Gottingen, 1982).
11. Searle, J. R. in *Semantics, an Interdisciplinary Reader in Philosophy, Linguistics and Psychology* (eds Steinberg, D. D. & Jakobovitz, L. A.) 134-141 (Cambridge University Press, 1971).
12. Warrington, E. K. & Shallice, T. *Brain* **107,** 829-853 (1984).
13. Warrington, E. K. & McCarthy, R. A. *Brain* **106,** 859-878 (1983).
14. Jackendoff, R., *Semantic and Cognition* (MIT Press, Cambridge, Massachusetts, 1983).

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Analysis of fusion gene and encoded photopigment of colour-blind humans

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IN humans, long-wavelength-sensitive and middle-wavelength-sensitive cone pigments are encoded by genes lying in a head-to-tail tandem array on the X chromosome. Deficiencies in red-green colour vision seem to arise from unequal recombination of these normal X-linked genes[1,2]. In some dichromats this recombination is believed to yield a fusion gene encoding a product with an absorption spectrum similar to that of one or the other of the normal photopigments[2]. Until now, however, such a relationship between the structure of a pigment gene and the spectral properties of its encoded pigment has not been directly shown. We have now sequenced a fusion gene isolated from a red-green colour-blind human and determined the spectral properties of the pigment that it encodes. The absorption spectrum of the photopigment was very similar to that of normal middle-wavelength-sensitive photopigment, even though about half of its DNA coding sequence seems to be derived from a gene encoding normal long-wavelength-sensitive pigment. These results indicate the regions of the X-encoded photopigment apoproteins that are responsible for differences in their spectral tuning, and imply that the striking variations in colour vision among anomalous trichromats of a particular type are not attributable to anomalous pigments with differing spectral peaks.

We established the colour vision of a 31-year-old male by psychophysical tests in which the appearances of mixtures of red and green primary lights were compared with the appearance of a standard yellow light (the Rayleigh match). He accepted

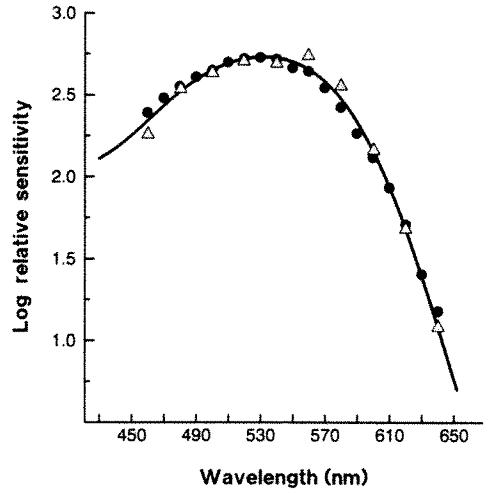

FIG. 1 Comparison of the spectral sensitivity of the MWS cone pigment from a protanope with the MWS cone pigment from a colour-normal human. ●, Protanope ERG sensitivity values corrected for pre-retinal absorption by the lens[12]. △, Spectral sensitivity of MWS cone pigment from a normal trichromat[6]. The wavelength of peak sensitivity for each was determined by translating a standard visual pigment absorption curve[13] on a log-wavenumber axis to obtain the best fit. The two pigment spectra have the same best-fitting curve (solid line; $\lambda_{max} = 530$ nm).

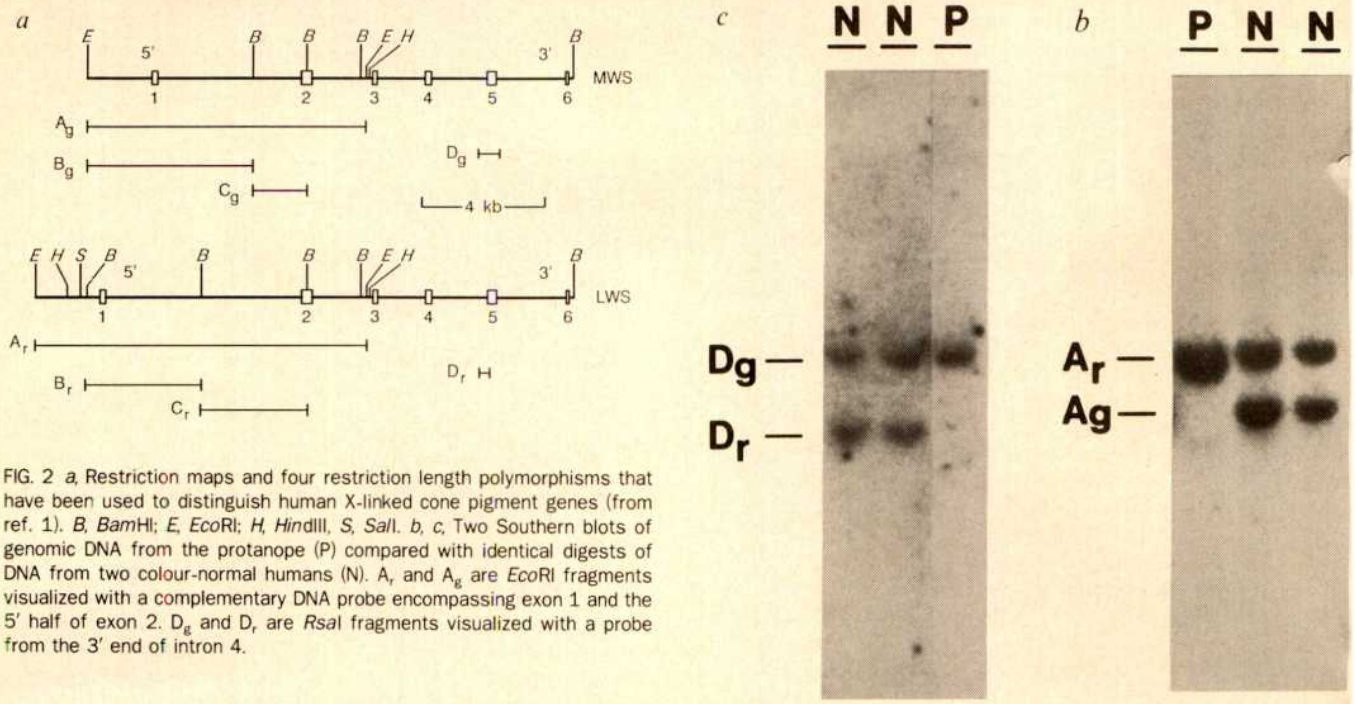

FIG. 2 *a*, Restriction maps and four restriction length polymorphisms that have been used to distinguish human X-linked cone pigment genes (from ref. 1). *B*, *Bam*HI; *E*, *Eco*RI; *H*, *Hin*dIII, *S*, *Sal*I. *b*, *c*, Two Southern blots of genomic DNA from the protanope (P) compared with identical digests of DNA from two colour-normal humans (N). A$_r$ and A$_g$ are *Eco*RI fragments visualized with a complementary DNA probe encompassing exon 1 and the 5' half of exon 2. D$_g$ and D$_r$ are *Rsa*I fragments visualized with a probe from the 3' end of intron 4.

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1
↓     614                 629                     644                     659                     674
GC CCC TTC GAA GGC CCG AAT TAC CAC ATC GCT CCC AGA TGG GTG TAC CAC CTC ACC AGT GTC TGG ATG
   Pro Phe Glu Gly Pro Asn Tyr His Ile Ala Pro Arg Trp Val Tyr His Leu Thr Ser Val Trp Met

                  689         *       704                     719                     734
   ATC TTT GTG GTC ACT GCA TCC GTC TTC ACA AAT GGG CTT GTG CTG GCG GCC ACC ATG AAG TTC AAG
   Ile Phe Val Val Thr Ala Ser Val Phe Thr Asn Gly Leu Val Leu Ala Ala Thr Met Lys Phe Lys
                   Ile

        749                     764                     779                     794
   AAG CTG CGC CAC CCG CTG AAC TGG ATC CTG GTG AAC CTG GCG GTC GCT GAC CTA GCA GAG ACC GTC
   Lys Leu Arg His Pro Leu Asn Trp Ile Leu Val Asn Leu Ala Val Ala Asp Leu Ala Glu Thr Val

   809                  824                     839                     854                     869
   ATC GCC AGC ACT ATC AGC ATT GTG AAC CAG GTC TCT GGC TAC TTC GTG CTG GGC CAC CCT ATG TGT
   Ile Ala Ser Thr Ile Ser Ile Val Asn Gln Val Ser Gly Tyr Phe Val Leu Gly His Pro Met Cys
                           Val                 Tyr

                                                2
           884                     899          ↓          914                     929
   GTC CTG GAG GGC TAC ACC GTC TCC CTG TGT GGG ATC ACA GGT CTC TGG TCT CTG GCC ATC ATT TCC
   Val Leu Glu Gly Tyr Thr Val Ser Leu Cys Gly Ile Thr Gly Leu Trp Ser Leu Ala Ile Ile Ser

       944                     959                     974                     989                    1004
   TGG GAG AGG TGG CTG GTG GTG TGC AAG CCC TTT GGC AAT GTG AGA TTT GAT GCC AAG CTG GCC ATC
   Trp Glu Arg Trp Leu Val Val Cys Lys Pro Phe Gly Asn Val Arg Phe Asp Ala Lys Leu Ala Ile
                   Met

                  1019                    1034                    1049                    1064
   GTG GGC ATT GCC TTC TCC TGG ATC TGG GCT GCT GTG TGG ACA GCC CCG CCC ATC TTT GGT TGG AGC
   Val Gly Ile Ala Phe Ser Trp Ile Trp Ala Ala Val Trp Thr Ala Pro Pro Ile Phe Gly Trp Ser

    3
    ↓  1079                    1094                    1109                    1124
   AGG TAC TGG CCC CAC GGC CTG AAG ACT TCA TGC GGC CCA GAC GTG TTC AGC GGC AGC TCG TAC CCC
   Arg Tyr Trp Pro His Gly Leu Lys Thr Ser Cys Gly Pro Asp Val Phe Ser Gly Ser Ser Tyr Pro

  1139                 1154                    1169                    1184                    1199
   GGG GTG CAG TCT TAC ATG ATT GTC CTC ATG GTC ACC TGC TGC ATC ACC CCA CTC AGC ATC ATC GTG
   Gly Val Gln Ser Tyr Met Ile Val Leu Met Val Thr Cys Cys Ile Thr Pro Leu Ser Ile Ile Val
                                                               Ile         Ala         Met

                                                4
            1214                    1229        ↓   1244                    1259
   CTC TGC TAC CTC CAA GTG TGG CTG GCC ATC CGA GCG GTG GCA AAG CAG CAG AAA GAG TCT GAA TCC
   Leu Cys Tyr Leu Gln Val Trp Leu Ala Ile Arg Ala Val Ala Lys Gln Gln Lys Glu Ser Glu Ser

     1274                    1289                    1304                    1319                    1334
   ACC CAG AAG GCA GAG AAG GAA GTG ACG CGC ATG GTG GTG GTG ATG GTC CTG GCA TTC TGC TTC TGC
   Thr Gln Lys Ala Glu Lys Glu Val Thr Arg Met Val Val Val Met Val Leu Ala Phe Cys Phe Cys
                                                               Ile Phe     Tyr     Val

                 1349                    1324                    1379                    1394
   TGG GGA CCA TAC GCC TTC TTC GCA TGC TTT GCT GCT GCC AAC CCT GGC TAC CCC TTC CAC CCT TTG
   Trp Gly Pro Tyr Ala Phe Phe Ala Cys Phe Ala Ala Ala Asn Pro Gly Tyr Pro Phe His Pro Leu
                   Thr                                             Ala

        1409                    1424                    1439                    1454
   ATG GCT GCC CTG CCG GCC TTC TTT GCC AAA AGT GCC ACT ATC TAC AAC CCC GTT ATC TAT GTC TTT
   Met Ala Ala Leu Pro Ala Phe Phe Ala Lys Ser Ala Thr Ile Tyr Asn Pro Val Ile Tyr Val Phe
                           Tyr

                  5
  1469            ↓
   ATG AAC CGG CAG
   Met Asn Arg Gln
```

FIG. 3 Sequence of exons 2, 3, 4, and 5 of the MWS pigment gene from a protanope. The locations of the five introns[1] are indicated by arrows. The numbering convention is that of Nathans *et al.*[1]. For the region outside the box (exons 4 and 5), the protanope gene sequence is identical to that of an identified normal MWS pigment gene fragment (gJHN21; ref. 1). For these two exons the underlined nucleotides in the protanope gene sequence are those that differ from corresponding nucleotides in a genomic LWS pigment gene fragment (gJHN33; ref. 1), and the lower amino-acid sequence is that of the identified normal LWS pigment. Exons 2 and 3 (enclosed in the box) of the protanope gene are identical to those of the identified LWS pigment genes in all of the nucleotide substitutions that distinguish the identified normal MWS from the LWS pigment genes[1]. But exons 2 and 3 of the protanope gene differ slightly from those of each of the three genes (one derived from a genomic library and two derived from a human retina cDNA library, no two of which were identical) that Nathans *et al.*[1] identified as encoding normal LWS pigment. For example, exons 2 and 3 of the protanope gene differ from those of the genomic LWS pigment gene fragment (gJHN53; ref. 1) by three nucleotides, but for two of these substitutions the protanope gene is identical to the other two LWS pigment genes, whereas the third is a silent nucleotide substitution (indicated by the asterisk) in which the protanope gene sequence differs from both MWS and LWS sequences. For exons 2 and 3 the nucleotides of the protanope gene that differ from those of a MWS gene fragment (gJHN21; ref. 1) are underlined, and the lower amino-acid sequence is that deduced from the MWS pigment gene (identical amino-acid residues are left blank). A genomic library derived from germ line DNA from the protanope was constructed[14] in the cosmid vector plasmid pWE15 (ref. 15). A fragment containing the protanope MWS pigment gene was identified by its hybridization with a cDNA clone probe. Sequences (both strands) were determined by the dideoxy chain termination method[16].

the red and green mixture of all proportions as matching the yellow light in both small-field (2 degrees) and large-field (11 degrees) versions of the test. The subject was thus a true dichromat[3,4]—a protanope, having only a single X-encoded cone pigment absorbing light of middle to long wavelengths.

We determined the absorption spectrum of this X-encoded cone pigment by analysing a retinal gross potential—the electroretinogram (ERG). To measure relative sensitivity, we adjusted the quantal intensity of a rapidly flickering monochromatic test light to produce a response that matched in amplitude the response to an interleaved flickering reference light[5]. Repetition of this procedure for many test wavelengths yields a curve that accurately characterizes absorption spectra of middle-wavelength-sensitive (MWS) and long-wavelength-sensitive (LWS) cone pigments in dichromatic subjects[5]. The derived sensitivity of the dichromat curve (Fig. 1, ●) thus gave the spectral sensitivity of this MWS pigment. Schnapf *et al.*[6] have measured the spectral sensitivity of normal human cones. The absorption spectra for the protanope MWS cone and the normal MWS cone were very similar (Fig. 1) and the spectral peaks were identical ($\lambda_{max} = 530$ nm).

Southern blots for two different restriction-enzyme digests of genomic DNA from the protanopic subject are shown in Fig. 2. For humans with normal colour vision, similar analysis reveals two hybridizing bands corresponding to two classes of X-linked pigment genes[1]. For the protanopic subject, we observed only a single hybridizing band for each digest, indicating the presence of a single X-linked pigment gene. The earlier identification of the normal pigment genes[1,2] (Fig. 2*a*) enabled us to identify this pigment gene as a fusion gene which had derived its 5′ end from a normal LWS pigment gene and its 3′ end from a normal MWS pigment gene.

We obtained a DNA fragment containing the entire X-linked photopigment gene of the protanope from a library constructed using total genomic DNA, and determined the sequences of exons 2, 3, 4 and 5. Comparison with the normal X-linked pigment genes[1] indicated that this fusion gene had derived its exons 2 and 3 from a normal LWS pigment gene and its exons 4 and 5 from a normal MWS pigment gene (Fig. 3). The fact that the absorption spectrum of the pigment encoded by the fusion gene does not differ from that of a normal MWS pigment indicates that the amino-acid substitutions encoded in exons 2 and 3 have little or no effect on the absorption spectrum of the X-linked photopigments. Nathans *et al.*[1] found no differences between exons 1 or between exons 6 of the MWS and LWS pigment genes. Thus the spectral differences between MWS and LWS pigments seem to be governed entirely by the amino-acid substitutions encoded in exons 4 and 5.

Except for several homologous substitutions[7] (which are not expected to alter the absorption spectrum of the chromophore) all the amino-acid differences between MWS and LWS photopigments that occur in the transmembrane segments encoded by exons 4 and 5 are substitutions of nonpolar groups for hydroxyl-bearing groups[1]. Studies of bacteriorhodopsin demonstrate that similar substitutions of nonpolar for polar groups (for example, Phe for Trp) can have dramatic effects on the absorption spectrum of the chromophore[8]. Depending on their position relative to the chromophore, hydroxyl groups have been proposed to preferentially stabilize or destabilize the ground state relative to the excited state and thus shift the absorption spectrum to the shorter or longer wavelengths[9]. Figure 4 shows the location of all the nucleotide differences responsible for non-homologous amino-acid substitutions that are located in the transmembrane segments encoded by exons 4 and 5. There are two such substitutions in exon 4 and three in exon 5. The most 5′ proximal of these nucleotide substitutions in exon 5 is marked by the restriction site polymorphism that distinguishes fragment D_r from D_g.

The locations and small number of nucleotide substitutions encoding the amino acids that are candidates for producing the spectral difference between X-linked pigments (Fig. 4) limits the number of different pigments with intermediate spectral peaks that can result from genetic recombination. These limitations have implications for understanding the genetic mechanisms underlying the most common forms of defective colour vision—the anomalous trichromacies. Nathans *et al.*[2] propose that the anomalous pigments result from recombination of the identified normal pigment genes. Because all protanomalous individuals that have been studied using Southern blot analysis have fusion genes containing fragment D_g, and all deuteranomalous subjects have fusion genes containing fragment D_r (refs 2, 10), both types of anomalous pigment genes must be produced by crossovers upstream of the D_g-D_r marker. The nucleotide substitutions that give rise to the two hydroxyl-bearing amino-acid substitutions in the MWS exon 4 are separated by only seven nucleotides, so recombination events in the region between them are expected to occur only rarely. These observations suggest that one or both of the substitutions encoded in exon 4 are responsible for the small spectral shift differentiating the X-linked pigments in anomalous trichromats, whereas one or more of the substitutions encoded in exon 5 are

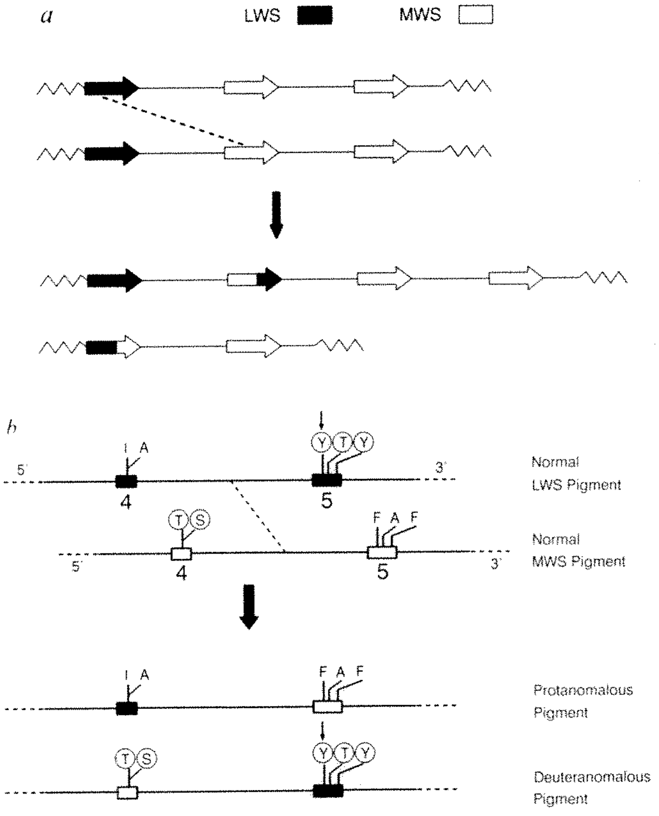

FIG. 4 *a*, Scheme showing how unequal intragenic recombination between normal MWS and LWS pigment genes can produce fusion genes. Each arrow represents a single gene (from ref. 2). *b*, Detail of *a*, showing only exons 4 and 5 of the pigment genes. The locations of nucleotide differences encoding amino-acid substitutions that are candidates for producing the spectral shifts differentiating the products of X-linked pigment genes are labelled according to the amino acid produced. The hydroxyl-bearing amino acids are circled (Y, tyrosine; T, threonine; S, serine), and their nonpolar counterparts are not circled (I, isoleucine; A, alanine; F, phenylalanine). The location of the *Rsa*I site that differentiates restriction fragment D_r from D_g (Fig. 1) is indicated by a small arrow. Cross-overs upstream of the two substitutions in exon 4 produce fusion genes that encode pigments with spectra unchanged from those of products encoded by the original genes. Exchanges in the region between the substitutions in exon 4 and those in exon 5 (such as the one illustrated) are proposed to produce two fusion genes, one that encodes a pigment that is slightly 'red-shifted' from the normal MWS pigment (protanomalous pigment) and a second that is slightly 'green-shifted' from the normal LWS pigment (deuteranomalous pigment).

responsible for the larger spectral shift that differentiates MWS from LWS pigments. It seems that, except for a crossover between the two exon 4 substitutions (Fig. 4), only a single spectrally intermediate protanomalous hybrid pigment and a single spectrally intermediate deuteranomalous pigment could be produced by exchanges upstream of the D_g-D_r marker.

Southern-blot analysis of pigment genes from colour-anomalous subjects does not seem to distinguish between individuals with mild and more extreme forms of anomalous trichromacy. Nathans *et al.*[2] suggest that different locations of crossing over can produce a variety of anomalous pigments with differing spectral peaks, and that this spectral variation accounts for the differences in colour-discrimination ability among anomalous trichromats. The results presented here indicate otherwise—that both mild and extreme forms of protanomalous colour vision are probably based on the same anomalous photopigment and that, similarly, a second anomalous pigment underlies both mild and extreme forms of deuteranomalous colour vision. This conclusion (1) is consistent with recent results from colour matching[3], which show that under conditions designed to maximize colour discrimination, both mild and extreme anomalous subjects make the same colour match and thus seem to have the same anomalous pigment; and (2) reinforces the conclusion reached from psychophysical studies[3,4,11] that tl differences in colour-discrimination ability within each of tl two classes of anomalous trichromats cannot be explained l differences in the absorption spectra of the underlying photopi ments.

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1. Nathans, J., Thomas, D. & Hogness, D. S. *Science* **232,** 193-202 (1986).
2. Nathans, J., Piantanida, T. P., Eddy, R. L., Shows, T. B. & Hogness, D. S. *Science* **232,** 203-210 (198
3. Nagy, A. L. *J. opt. Soc. Amer.* **72,** 571-577 (1982).
4. Pokorny, J. & Smith, V. C. *Color Res. Appl.* **7,** 159-164 (1982).
5. Neitz, J. & Jacobs, G. H. *J. opt. Soc. Amer.* **A1,** 1175-1180 (1984).
6. Schnapf, J. L., Kraft, T. W. & Baylor, D. A. *Nature* **325,** 439-441 (1987).
7. Lehninger, A. L. *Biochemistry* (Worth, New York, 1975).
8. Hackett, N. R., Stern, L. J., Chao, B. H., Kronis, K. A. & Khorana, H. G. *J. biol. Chem.* **262,** 9277-92 (1987).
9. Kosower, E. M. *Proc. natn. Acad. Sci. U.S.A.* **85,** 1076-1080 (1988).
10. Drummond-Borg, M., Deeb, S. & Motulsky, A. G. *Am. J. hum. Genet.* **43,** 675-683 (1988).
11. Hurvich, L. M. & Jameson, D. *Mod. Probl. Opthalmol.* **13,** 200-209 (1974).
12. Wyszecki, G. & Stiles, W. S. *Color Science* (Wiley, New York, 1982).
13. Dawis, S. M. *Vision Res.* **21,** 1427-1430 (1981).
14. DiLella, A. G. & Woo, S. L. C. *Meth. Enzym.* **152,** 199-212 (1987).
15. Wahl, G. M. *et al. Proc. natn. Acad. Sci. U.S.A.* **84,** 2160-2164 (1987).
16. Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74,** 5463-5467 (1977).

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Characterization of a naturally processed MHC class II-restricted T-cell determinant of hen egg lysozyme

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COMPELLING evidence indicates that T cells recognize complexes formed by major histocompatibility complex-encoded molecules and antigenic peptide fragments[1-3]. This is based largely on the ability of small synthetic peptides to substitute for naturally processed antigen in stimulating T cells. Naturally processed fragments of exogenous antigen are thought to arise by limited proteolytic degradation of native antigen inside acidic compartments of antigen-presenting cells[3-6], but until now no physiologically processed antigen has been directly analysed. Here we report the characterization of physiologically processed antigen eluted from mouse class II major histocompatibility complex I-E^d molecules. The antigenic material corresponds to a previously described antigenic determinant of hen egg lysozyme (HEL 107-116) and has a relative molecular mass M_r of about 2,000. HPLC analysis identified at least two or three separate molecular species, suggesting limited, albeit significant, heterogeneity of naturally processed peptides. Finally, under our experimental conditions, it was calculated that a substantial proportion (10-40%) of I-E^d molecules were occupied by these HEL-derived antigenic determinants.

Taking advantage of the high stability of the complexes formed between major histocompatibility complex (MHC) molecules and antigenic peptides, we decided to attempt the isolation and characterization of naturally processed HEL peptides from HEL-pulsed A20-1.11 B lymphoma cells (A20 cells). As a first step, we determined whether complexes containing antigenic determinants could be identified in a preparation of affinity-purified class II MHC molecules. For this purpose, I-E^d molecules were purified from a lysate of HEL-pulsed A20 cells (HEL/I-E^d), and inserted into planar membranes[8,9]. These structures strongly stimulated interleukin-2(IL-2) production by the I-E^d-restricted T cell hybridoma 1 H-11.3, for which the sequence HEL 107-116 has previously been identified as th minimal antigenic determinant[7]. Thus, this I-E^d preparatio carried naturally processed material corresponding to the HEI 107-116 determinant (Table 1, first row).

TABLE 1 Isolation of naturally processed antigenic material from antige pulsed B lymphoma cells

Source of I-E^d	Antigen added to purified I-E	IL-2 (U ml)$^{-1}$ produced by 1 H-11.3 T cells
HEL-pulsed A20	Nil	1,280
Unpulsed A20	Synthetic HEL 107-116 (20 ng)	20
Unpulsed A20	Synthetic HEL 107-116 (200 ng)	640
Unpulsed A20	Acid-eluted peptides from HEL/I-E^d	80

A20 cells were grown in RPMI medium supplemented with 5% fetal ca serum, 100 μg ml^{-1} streptomycin, 100 U ml^{-1} penicillin G, and 5 × 10 β-mercaptoethanol. A20 cells were pulsed for 24 h with 1 mg ml^{-1} HE (Grade I, Sigma). I-E^d molecules were purified from both pulsed and unpulse cells by affinity chromatography on 14.4.4 anti-I-E^d monoclonal antibod column[8]. To prepare the acid-eluted peptides, I-E^d (350 μg) was purifie from 1.5 × 10^{10} HEL-pulsed A20 cells (HEL/I-E^d), acetonitrile-precipitate treated for 30 min at 37 °C with 2.5 M acetic acid, and then loaded on Sephadex G-10 column equilibrated with acetate buffer[11]. The peptid material eluted in the void volume was collected and lyophilized twic Complexes between I-E^d and peptides prepared *in vitro* were obtained b dissolving the lyophilized peptides in 10 μl phosphate-buffere saline/100 mM Tris-HCl, pH 7.2, supplemented with 1 mg ml^{-1} sodium azid and a cocktail of protease inhibitors (16 mM EDTA, 2.6 mM 1,10-phenanthrc line, 0.15 mM pepstatin A and 2 mM PMSF) (ref. 10), and 10 μl of a 1 2 mg ml^{-1} I-E^d preparation. After incubating for 1 d at room temperatur the samples were diluted to 250 μl with PBS containing 1 mg ml^{-1} sodiur azide, 1% *n*-octyl-β-D-glucopyranoside (Sigma), 175 μg ml^{-1} L-α-phos phatidylcholine (Sigma), and 25 μg ml^{-1} cholesterol (Sigma). I-E^d-peptid complexes were inserted into planar membranes and tested in a T-ce stimulation assay as described[8,9].

The next step in this series of experiments would be to dissoci ate the antigenic determinants from the I-E^d molecules an biochemically characterize them. For this purpose, it wa necessary to devise as sensitive an assay as possible. To decreas the amount of antigenic material required to trigger IL-2 releas by the 1 H-11.3 T cell hybridoma, we formed I-E^d/peptid complexes *in vitro*, under conditions already established a optimal[10], and used these complexes, inserted into planar mem-

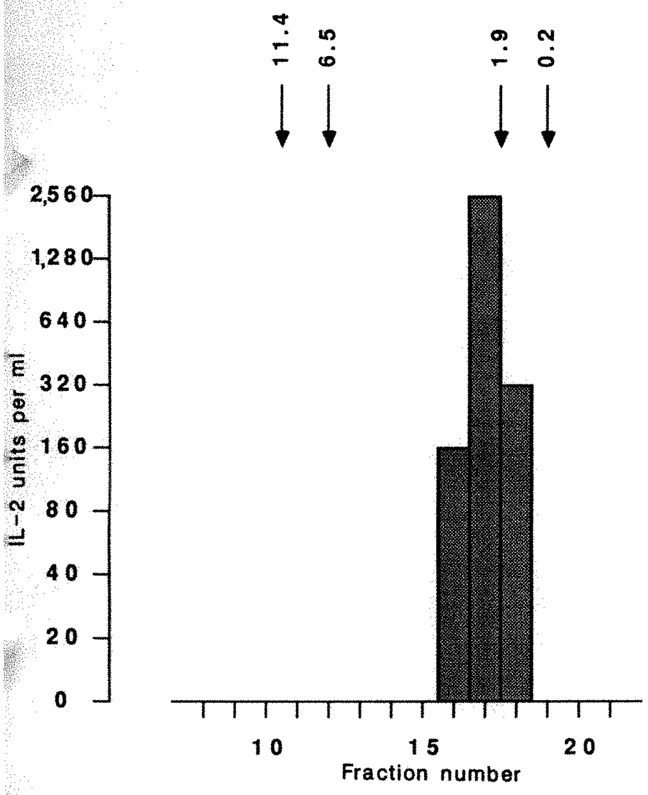

FIG. 1 Gel filtration analysis of a naturally processed I-E^d-restricted determinant of lysozyme.

METHODS. Acid-eluted material obtained from 600 μg HEL/I-E^d complexes (Table 1) was loaded on a Sephadex G-50 column (1.5 × 90 cm) equilibrated with 60 mM ammonium acetate buffer, pH 4.8 (ref. 11). Fractions (8 ml) were collected and lyophilized twice. One-fifth of each fraction was tested as described in the legend to Table 1. The positions of molecular weight markers are indicated (M_r 11.4 K, cytochrome *c*; 6.5 K, aprotinin; 1.9 K, Y-HEL 105–120; 0.2 K, $Cr_2O_7^{2-}$). In this experiment, I-E^d complexes prepared with 20 ng and 200 ng synthetic HEL 107–116 peptide stimulated 1 H-11.3 T cells to produce 1,280 U ml^{-1} IL-2 and >2,560 U ml^{-1} IL-2, respectively.

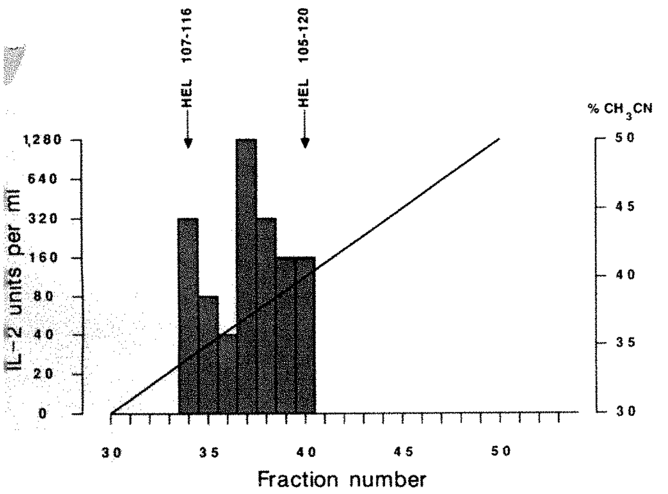

FIG. 2 Reverse-phase HPLC analysis of a naturally processed I-E^d-restricted determinant of lysozyme.

METHODS. Acid-eluted material obtained from 350 μg HEL/I-E^d complexes (Table 1) was separated on a μ-Bondapak C-18 reverse-phase HPLC column (Waters System) by forming a linear gradient of 0.08% trifluoroacetic acid in water and 0.08% trifluoroacetic acid in acetonitrile (1% per min). Fractions (1 ml) were collected, lyophilized, and tested as described in the legend to Table 1. The elution positions of the synthetic HEL 107–116 and HEL 105–120 peptides are indicated.

branes, in the T cell hybridoma stimulation assay[8,9]. Following this approach, we could detect as little as 20 ng HEL 107–116 peptide (Table 1), whereas, if directly added to antigen-presenting cells, 250 ng were required to reach similar levels of T-cell stimulation (ref. 7, and data not shown).

A crude preparation of peptides bound to HEL/I-E^d complexes was obtained by acid treatment of acetonitrile-precipitated HEL/I-E^d material, followed by Sephadex G-10 chromatography[11]. As assessed by SDS-gel electrophoresis, this step completely depleted the acid-eluted HEL/I-E^d peptide preparation of I-E^d molecules (data not shown). The acid-eluted HEL/I-E^d peptides were then incubated with I-E^d molecules purified from A20 cells grown in the absence of any HEL material, and the putative complexes were inserted into planar membranes as already described. These antigen-presenting structures stimulated IL-2 release from 1 H-11.3 T cells, indicating that peptide material containing the HEL 107–116 sequence had been eluted from the I-E^d isolated from cells incubated with HEL, and that these peptides could be rebound to I-E^d molecules (Table 1, last row). Similar data were obtained in the case of an I-A^d-restricted and ovalbumin 323–339 peptide-specific T cell hybridoma (data not shown).

To initiate the characterization of this acid-eluted HEL/I-E^d material, the molecular weight was estimated by gel filtration on a Sephadex G-50 column. Each fraction was tested for its capacity to produce, when recombined to I-E^d, complexes antigenically active for 1 H-11.3 T cells. Stimulatory material was eluted in a single homogeneous peak, with an apparent relative molecular mass of ~2,000 (M_r 2K) (Fig. 1).

To further characterize the naturally processed HEL-derived material, an acid-eluted peptide preparation was subjected to reverse-phase HPLC and each fraction was tested for its capacity, when incubated with I-E^d, to form T-cell stimulatory complexes. Material antigenic for 1 H-11.3 T cells was eluted between 34–40% acetonitrile. The activity profile suggested that at least two or three peptide species were produced by A20 cells during processing of HEL molecules (Fig. 2). The synthetic peptides HEL 107–116 and HEL 105–120 eluted at 34% and 40% acetonitrile, respectively, suggesting that the HEL fragments generated by A20 cells are not grossly dissimilar from these peptides.

To evaluate the possibility that limited degradation of the antigenic material occurred during the isolation procedure, a radioiodinated synthetic peptide corresponding to [^{125}I]Y-HEL 105–120 was purified as described. No degradation could be detected by reverse-phase HPLC, indicating that the antigenic HEL peptides that we characterized were representative of those peptides produced by processing in A20 cells (data not shown).

This assay allowed us to estimate directly the proportion of I-E^d molecules on HEL-pulsed A20 cells that were occupied by peptides containing the HEL 107–116 determinant detected by 1 H-11.3. We therefore compared the antigenicity of an I-E^d preparation from HEL-pulsed A20 (HEL/I-E^d) with synthetic HEL 107–116/I-E^d complexes prepared *in vitro*. The amount of synthetic HEL 107–116 peptide bound to I-E^d molecules was first determined by Scatchard analysis, as previously described[10]. Thus it was calculated that under saturating concentrations of HEL 107–116 peptide (>10 μg ml^{-1}), 7.5% of I-E^d molecules were occupied by this peptide. Then various amounts of synthetic HEL 107–116/I-E^d complexes were inserted into planar membranes, and the capacity to stimulate 1 H-11.3 T cells was measured for each (Fig. 3). The total amount of I-E^d molecules inserted into each planar membrane sample was kept constant by adding I-E^d molecules purified from unpulsed A20 cells, to obtain a direct correlation between the T-cell response and the density of antigenic complexes borne by the planar membranes. The response to the synthetic HEL 107–116 peptide/I-E^d complexes was then compared with naturally processed HEL. It was found that HEL/I-E^d complexes inserted at 16 nM into planar membranes stimulated 1 H-11.3 T cells to produce 640 U ml^{-1}

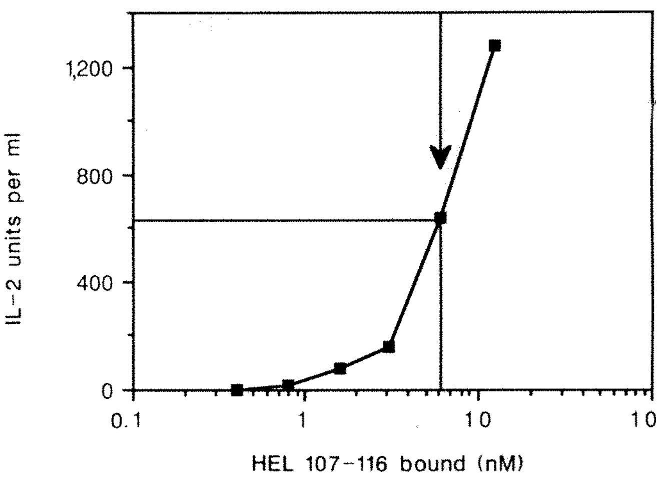

FIG. 3 Occupancy of I-E^d molecules purified from HEL-pulsed B lymphoma cells by HEL 107–116-containing determinants. To generate a standard curve, various concentrations of synthetic HEL 107–116 peptide bound to I-E^d molecules and inserted into planar membranes were tested for their ability to trigger 1 H-11.3 T cell stimulation. Then a known concentration of I-E^d purified from HEL-pulsed A20 cells (16 nM) was inserted into planar membranes and used to stimulate 1 H-11.3 T cells. An equivalent stimulation to 6 nM of synthetic peptide HEL 107–116/I-E^d complexes was obtained. METHODS. The concentration (nM) of purified I-E^d molecules able to bind antigen was determined by Scatchard analysis[10]. The amount of synthetic HEL 107–116 peptide necessary to fully saturate the same I-E^d molecules was measured by testing a dose range of synthetic HEL 107–116 peptide for its capacity to abrogate binding of ^{125}I-radiolabelled λ-repressor 12–26 peptide[10]. I-E^d molecules (0.5 mg ml^{-1}) were then incubated with 1 mg ml^{-1} synthetic HEL 107–116 peptide (30-fold excess over the saturating dose), using the same conditions as in the binding assay (see Table 1 legend). After the incubation, I-E^d molecules were recovered from free peptide by passage over a Sephadex G-50 column and inserted into planar membranes[8,9]. The total amount of I-E^d molecules inserted was kept constant (10–25 μg ml^{-1}) by adding I-E^d molecules purified from unpulsed A20 cells. In parallel, I-E^d molecules were prepared from HEL-pulsed A20 cells, inserted into planar membranes, and tested for 1 H-11.3 T cell stimulation. As before, I-E^d concentration was adjusted at ~25 μg ml^{-1} by the addition of I-E^d purified from unpulsed A20 cells.

IL-2. A similar T-cell activation was reached with 6 nmol synthetic HEL 107–116 peptide bound to I-E^d molecules (Fig. 3). Assuming that HEL 107-116/I-E^d complexes were as stimulatory as naturally processed HEL/I-E^d complexes, these data would indicate that 38% of the I-E^d molecules expressed by HEL-pulsed A20 cells were occupied by HEL 107-116-like determinants.

An independent estimate of the extent of occupancy was based on the amount of antigenic peptides eluted from an HEL/I-E^d preparation after its separation by G-50 gel filtration separation. In this experiment, antigenic material obtained from 2 nmoles of I-E^d yielded T-cell stimulatory activity corresponding to 2,000–3,000 U ml^{-1} IL-2 (Fig. 1). Similar levels of IL-2 production were attained using ~0.2 nmol synthetic HEL 107-116 peptide. So by this calculation it seems that ~10% of the I-E^d molecules purified from HEL-pulsed A20 cells carried HEL 107-116 determinants. This value is probably a minimal estimate as the G-50 fractions would also be expected to contain non-HEL-derived I-E^d-binding peptides which would tend to compete with and lower the efficiency of binding of the HEL peptides to I-E^d (ref. 11).

In conclusion, the preparation of complexes between MHC molecules and antigenic peptides allowed us to characterize a naturally processed determinant of HEL. The heterogeneity of the antigenic peptides produced by A20 cells may be due to cleavages at different positions in the HEL sequence, reflecting the use of either different proteases or proteases displaying a broad substrate specificity.

Quantitation of the proportion of I-E^d from HEL-pulsed A20 cells bearing HEL 107-116-containing determinants gave surprisingly high estimates (10–40%). These data can be rationalized, at least in part, by considering that HEL represented, under our experimental conditions, about one-third of the total extracellular protein (1 mg ml^{-1} HEL and 2–3 mg ml^{-1} of fetal calf serum proteins). Furthermore, the HEL 107-116 peptide region is the chief I-E^d binding site of HEL (ref. 7). We cannot exclude the possibility, however, that there are covalent modifications of antigenic material which increase its antigenic potency[12]. Direct sequencing of naturally processed antigenic peptides should solve these issues. □

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1. Shimonkevitz, R., Colon, S., Kappler, J. W., Marrack, P. & Grey, H. M. *J. Immun.* **133,** 2067–2074 (1984).
2. Babbitt, B., Allen, P., Matsueda, G., Haber, E. & Unanue, E. *Nature* **317,** 359–361 (1985).
3. Buus, S. *et al. Proc. natn. Acad. Sci. U.S.A.* **83,** 3968–3971 (1986).
4. Chesnut, R. W., Colon, S. M. & Grey, H. M. *J. Immun.* **129,** 2382–2387 (1982).
5. Ziegler, K. & Unanue, E. R. *J. Immun.* **127,** 1869–1875 (1981).
6. Streicher, H. Z., Berkower, I. J., Busch, M., Gurd, F. R. N. & Berzofsky, J. *Proc. natn. Acad. Sci. U.S* **81,** 6831–6835 (1984).
7. Adorini, L. *et al. Proc. natn. Acad. Sci. U.S.A.* **85,** 5181–5185 (1988).
8. Coeshott, C. M. *et al. J. Immun.* **136,** 2832–2838 (1986).
9. Buus, S., Sette, A., Colon, S. M., Jenis, D. M. & Grey, H. M. *Cell* **47,** 1071–1077 (1986).
10. Sette, A., Buus, S., Colon, S., Miles, C. & Grey, H. M. *J. Immun.* **142,** 35–40 (1989).
11. Buus, S., Sette, A., Colon, S. M. & Grey, H. M. *Science* **242,** 1045–1047 (1988).
12. Falo, L. D. Jr., Benacerraf, B. & Rock, K. L. *Proc. natn. Acad. Sci. U.S.A.* **83,** 6994–6997 (198

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Sequence and expression of a frog brain complementary DNA encoding a kainate-binding protein

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EXCITATORY amino acids (EAAs) are important neurotransmit ters in the vertebrate central nervous system[1]. Electrophysiologica and ligand-binding studies indicate that at least three differen receptor subtypes for EAAs exist—*N*-methyl-D-aspartate, kainat and quisqualate receptor subtypes—on the basis of the preferre agonist of the receptors[1,2]. We recently purified a kainate-bindin protein (KBP) from frog (*Rana pipiens berlandieri*) brain b domoic acid (a high-affinity kainate analogue) affinit

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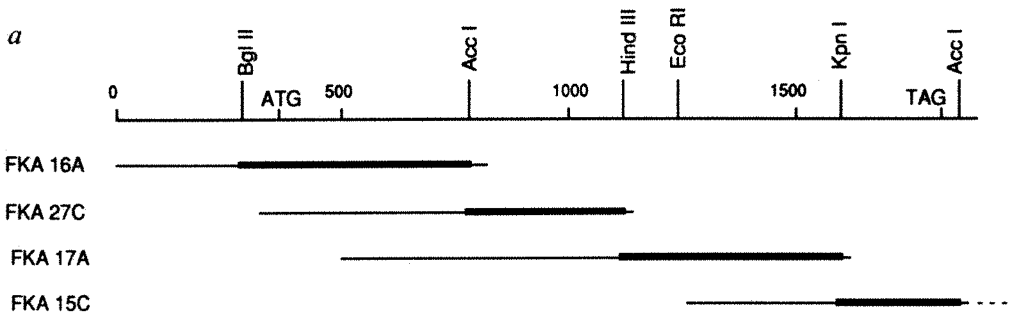

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        10                  30                  50                  70                  90
AGATCTCTCCTCTTGCATTGCTGTTCAGGAGTGAGTAAGAGACGGAGGAGGCTCAAACACTGCTGGAAAAAAATATACAGCCATGGAGAAAGCCCTAATG
                                                                                  M  E  K  A  L  M      6
        110                 130                 150                 170                 190
CTGTTTTTGGCAGTTTCCCTCCTCTCCTTGGGTCACACAGATGGTAAAGAAAATGCCGAAACTTTGTTAAAGGAAAGAACAAAACGTCAAATTCCAAAAACT
 L  E  L  A  V  S  L  L  S  L  G  H  T  D  G  K  E  N  A  E  T  L  L  K  E  R  T  K  R  Q  I  P  K  T    40
   210                 230                 250                 270                 290
CTGACTGTCACCACAATTTTGGAAAAGCCTTTTGCGATGAAGACTGAATCAGATGCCTTGGAAGGATATGCCATTGATCTTCTCTCTGAGCTCACTCAGTCT
 L  T  V  T  T  I  L  E  K  P  F  A  M  K  T  E  S  D  A  L  E  G  Y  A  I  D  L  L  S  E  L  T  Q  S    74
  310                 330                 350                 370                 390
CTGGGATTCAATTACACATTGCATATAGTGAAGGATGGAAAATATGGATCCAAAGACCAGGAAGGAAACTGGAGTGGAATGGTGGGAGAAATCATCCGAAAG
 L  G  F  N# Y  T  L  H  I  V  K  D  G  K  Y  G  S  K  D  Q  E  G  N# W  S  G  M  V  G  E  I  I  R  K   108
 410                 430                 450                 470                 490                 5
GAAGCTGATTTGGCCATAGCACCTTTAACAATTACATCTGTGAGGGAGAATGCCATATCCTTCACCAAACCCTTCATGCAGACTGGGATTGGTATACTCCTA
 E  A  D  L  A  I  A  P  L  T  I  T  S  V  R  E  N  A  I  S  F  T  K  P  F  M  Q  T  G  I  G  I  L  L   142
10                  530                 550                 570                 590                 610
AAGAAAGATACAGCCGCTGAGAGCTCATACATGTTTGGTTTTCTGAACCCTTTCAGCAAGGAATTGTGGATAGGAATCATTATATCCTATGTGATCACCAGC
 K  K  D  T  A  A  E  S* S* Y* M* F* G* F* L* N* P* F* S* K* E* L* W* I* G* I* I* I* S* Y* V* I* T* S   176
                630                 650                 670                 690                 710
CTCTGCCTGTTTCTTGTTGGAAGGTTAAGTCCCTGTGAATGGACTGAACCCGCCAGTGAACAGAATCAGTTCACTCTATTGAACAGCCTTTGGTATGGAGTT
 L  C  L  F  L  V  G  R  L  S  P  C  E  W  T  E  P  A  S  E  Q  N  Q  F  T  L  L  N  S  L  W  Y  G  V   210
              730                 750                 770                 790                 810
GGAGCATTAACACTGCAAGGTGCCGAACCTCAGCCTAAAGCCTTATCTGCCCGGATCATCGCTGTCATTTGGTGGGTGTTTTCCATCACCCTCCTGGCTGCC
 G  A  L  T  L  Q  G  A  E  P  Q  P  K  A  L  S  A  R  I  I  A  V  I  W  W  V  F  S  I  T  L  L  A  A   244
             830                 850                 870                 890                 910
TACATTGGAAGCTTTGCTTCATACATTAACAGCAACACCAACCAAACTCCAAATATTCAGTCCGTAGAGGATTTGCTGAAACAAGATAAACTTGACTTTGGG
 Y  I  G  S  F  A  S  Y  I  N  S  N  T  N  Q  T  P  N  I  Q  S  V  E  D  L  L  K  Q  D  K  L  D  F  G   278
            930                 950                 970                 990                 1010
ACATTGAGCAACTCTTCCACTTTAAATTTCTTTAAGAATTCTAAGAATCCAACATTCCAAATGATCTATGAGTACATGGACAAGAGGAAGGACCGTGTGCTT
 T  L  S  N  S  S  T  L  N  F  F  K  N  S  K  N  P  T  F  Q  M  I  Y  E  Y  M  D  K  R  K  D  R  V  L   312
      1030                1050                1070                1090                1110
GTGAAAACTTTTTCTGAAGGAGTACAGCGAGTCCGAGAGTCAAACTATGCCTTCCTGGGAGAGTCCATCAGCCAAGACTTTGTGGTGGCCAAACACTGTGAC
 V  K  T  F  S  E  G  V  Q  R  V  R  E  S  N  Y  A  F  L  G  E  S  I  S  Q  D  F  V  V  A  K  H  C  D   346
     1130                1150                1170                1190                1210
CTTATTCGCGCCCCAGAAATGATTGGAGGCAGAGGATATGGCATCGCAGCAGAATTAGACTCCCCCCTGATCAGACCTCTGACTATCGCCATTTTGGAGCTT
 L  I  R  A  P  E  M  I  G  G  R  G  Y  G  I  A  A  E  L  D  S  P  L  I  R  P  L  T  I  A  I  L  E  L   380
    1230                1250                1270                1290                1310
TTTGAATCCGGAAAGCTGGAGTACCTGCGTCAGAAGTGGTGGGAAAACACCTGCTCTACACAAGACCAGACAGGTTGGGTACCCGTACAACCTCACACTTTG
 F  E  S  G  K  L  E  Y  L  R  Q  K  W  W  E  N  T  C  S  T  Q  D  Q  T  G  W  V  P  V  Q  P  H  T  L   414
  1330                1350                1370                1390                1410
GGCGGCATCTTCCTTATCCTGGGTATTGGCTTGGCTCTAGGGTTGATTGTCTCCTTCATGGAGCTCATGTGTAAATCAAGGAGCAATGCGGAACAACAGAAG
 G  G  I  F  L  I  L  G  I  G  L  A  L  G  L  I  V  S  F  M  E  L  M  C  K  S  R  S  N  A  E  Q  Q  K   448
1430                1450                1470                1490                1510                15
AAGTCCTGCTGCTCTGCATTCTCAGAGGAGATCGCTCAGCGCTTTGGAAAAACACAAAATCAGGAAGGTCTGGAAAAGAAATCCCCTACTTCGAACAGCTGT
 K  S  C  C  S  A  F  S  E  E  I  A  Q  R  F  G  K  T  Q  N  Q  E  G  L  E  K  K  S  P  T  S  N  S  C   482
30                  1550                1570
GATGAAGTGAAGGCATAGACCCGTGAAGCTCAGACCACTTACCTTGGTGTATAC
 D  E  V  K  A
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FIG. 1 *a*, Restriction enzyme map of cDNA clones encoding the KBP. Thick lines represent regions that are used to construct an expressible cDNA containing an entire open-reading frame. Broken line shows the regions not sequenced. Positions of the initiator methionine codon and stop codon of an open reading frame are shown by ATG and TAG, respectively. *b*, Nucleotide and deduced amino-acid sequence (single-letter code) of the KBP cDNA. Only the sequences used for the expression studies are shown. Nucleotides and amino acids are numbered in order, starting with the first residues. Amino-acid residues with asterisks show the chemically obtained KA2 sequence. Individually underlined amino-acid residues show the predicted signal peptide sequence[10]. Underlined sequences indicate the putative transmembrane domains predicted by regional hydropathy analysis[16,17]. Potential *N*-linked glycosylation sites[11] are indicated by #.

METHODS. KBP was isolated from frog brain by domoic-acid affinity chromatography[3]. The protein was further purified by SDS-PAGE. The main band migrating to a position corresponding to an M_r of 48,000 was excised, digested with *S. aureus* V8 protease in a stacking gel, and fractionated by electrophoresis on a 15% SDS-polyacrylamide gel. The gel was electroblotted onto an Immobilon membrane (Millipore, Bedford, Massachusetts). After staining with Coomassie blue, pieces containing the main digested fragments were excised and microsequenced. One long amino-acid sequence (KA2) was obtained from one of the fragments (single-letter code): SSYMFGFLNPFSKELXIGIIISYVIT, where X is an unidentified residue. Sense and antisense 20-base oligonucleotides were made using all possible codon combinations encoding the sequence near the N and C termini of KA2, respectively: 5′-TAYATGTTYGGNTTYYTNAA-3′, 5′-ACRTANSWDATDATDATNCC-3′. R, A or G; S, C or G; W, A or T; Y, C or T; D, A or G or T; and N, A or C or G or T. PCR (ref. 8) was performed using the oligonucleotides as primers and frog liver genomic DNA as a template. Frog liver genomic DNA was prepared by a standard method[24]. Annealing reactions were performed at 50 °C for 2.5 min, followed by a 3.5-min extension at 72 °C and 1-min denaturation at 94 °C. After 35 rounds of amplification, PCR-extended fragments of 65-bp were purified, subcloned into the *Sma*I site of the plasmid pGEM 3Z (Promega, Madison, Wisconsin) and sequenced[25]. Based on this resultant sequence, 65-base oligonucleotides were prepared and used to screen a frog brain cDNA library (Clontech, Palo Alto, California) in λgt11 vector: 5′-TACATGTTCGGGTTTCTGAACCCTTTCTCCAAGGAGCTGTGTATCGGCATCATCATAACCTACGT-3′. Hybridization and washing of filters were carried out according to a standard method[24]. The isolated cDNAs were subcloned into the plasmid pBluescript KS (+) (Stratagene, La Jolla, California) and sequenced[25]. Some nucleotide sequence differences were found between the PCR product and the KBP cDNA. These could have been due to replication errors of the reverse transcriptases and DNA polymerases used, and/or to genetic polymorphism among frogs.

chromatography[3], and showed that the kainate-binding activity was associated with a protein of relative molecular mass 48,000 (M_r 48 K)[3,4]. The pharmacological properties and the anatomical distribution of KBP were consistent with those of a kainate receptor–ionophore complex[3–5]. We have now isolated a complementary DNA encoding KBP of M_r 48 K. The deduced amino-acid sequence of the KBP has similar hydrophobic profiles to those found in other ligand-gated ion channel subunits[6], and shows some amino-acid sequence similarities to the corresponding regions of brain nicotinic acetylcholine receptor subunits[7]. Localization of the KBP messenger RNAs by *in situ* hydridization histochemistry is compatible with the results of immunohistochemistry and receptor autoradiography studies[5]. COS-7 cells transfected with the cDNA encoding the KBP show high-affinity kainate-binding activity with pharmacological properties similar to those of the biochemically purified KBP (ref. 3). These results provide the first molecular characterization of an EAA-binding site and raise the possibility that the KBP cDNA encodes a ligand-binding subunit of a kainate receptor–ionophore complex.

A sequence of 26 amino-acid residues (referred to as KA2), as well as other shorter sequences, was obtained from the peptide fragments of the purified KBP which had been digested with *Staphylococcus aureus* V8 protease. Because none of the peptide sequences contained a suitable region to make oligonucleotide probes with little degenerativity, we used the polymerase chain reaction (PCR) procedure[8] to obtain a precise nucleotide sequence for KA2 (see Fig. 1 legend for experimental details). A fragment of genomic DNA of the expected length was amplified from a pool of frog liver genomic DNA. Sequenc analysis of the fragment revealed a nucleotide sequence tha encoded the KA2 sequence between the two regions used t prepare the primers. The revealed nucleotide sequences and th primer sequences in the PCR product were used to make a lon oligonucleotide probe to screen a frog brain cDNA library i λgt11 vector. From 7×10^5 recombinants, four clones were isolated and used to obtain longer cDNA clones. Four overlappin longer clones (FKA16A, FKA27C, FKA17A and FKA15C) wer obtained from a total of 1.4×10^6 recombinations and studie further (Fig. 1*a*).

Sequence analysis of these clones showed a 1,461-base-pai (bp) open-reading frame potentially encoding 487 amino-aci residues (Fig. 1*b*). The sequence around the predicted initiato methionine codon (ATG) agrees with the consensus sequenc described by Kozak[9]. The deduced amino-acid sequence show a hydrophobic segment at the N terminus, which probabl encodes a signal peptide. The N terminus of the mature protein was predicted by the method of von Heijne[10]. The proposed mature protein is preceded by a signal peptide of 17 residues and composed of 470 amino-acid residues with a calculated M of 52.5 K. Two potential *N*-linked glycosylation sites[11] are found at position 78 and 96 in the putative N-terminal extracellular segment. The amino-acid sequence of the predicted mature protein contains the KA2 sequence at residues 150–175. Other shorter amino-acid sequences obtained by the microsequencing of the V8 protease-treated fragments are also found at residues 57–66, 62–74, 110–125 and 353–366.

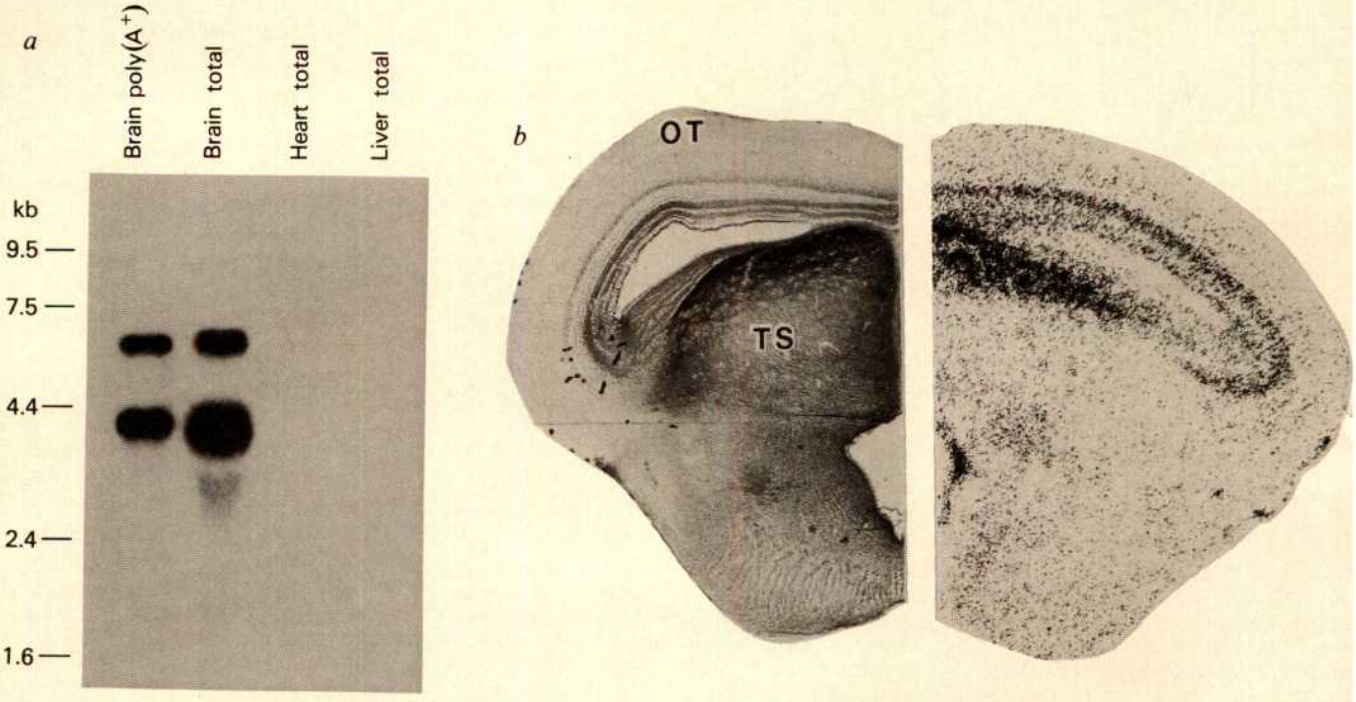


FIG. 2 *a*, Northern-blot analysis of the KBP mRNA. *b*, Comparison of labelling pattern in frog brain with immunostaining of KBP (left) and *in situ* hybridization histochemistry of KBP mRNA (right). Coronal sections of mesencephalon and optic tectum are presented. TS, Torus semicircularis; OT, Optic tectum.

METHODS. *a*, Frog brain total and poly(A)$^+$ RNA, and frog heart and liver total RNAs were prepared using a standard method[26]. Each total RNA (10 μg) and 0.5 μg poly(A)$^+$ RNA were electrophoresed on a 1% formaldehyde-agarose gel and blotted onto a nitrocellulose membrane. Hybridization was carried out in a buffer containing 5×SSC and 50% formamide at 42 °C. The pBluescript plasmid containing the expressible KBP cDNA sequence was used as a probe. The membrane was washed with 2×SSC at 55 °C and exposed overnight. The same pattern of signals was obtained after a very high stringency wash with 0.1×SSC at 70 °C. *b*, Immunohistochemistry was performed as previously described[5]. After incubating with the monoclonal antibody, KAR-A1 (refs 4, 5), immunostaining was visualized with avidin–biotin–peroxidase complex. *In situ* hybridization histochemistry was performed on freshly frozen frog brain sections. Sections (15 μm) were cut on a cryostat and mounted onto gelatin- and poly-L-lysine-coated slides. After fixation in phosphate buffer containing 4% paraformaldehyde for 10 min at 4 °C followed by rinsing in 2×SSC, sections were hybridized with ^{35}S-labelled oligonucleotide probes overnight in buffer containing 5×SSC and 50% formamide at 37 °C. Two 48-base oligonucleotides complementary to the sequences at residues 421–468 and 469–516 (Fig. 1) were used as probes. After hybridization, slides were washed in 0.5×SSC at 40 °C, dehydrated and exposed to X-ray films for 20 days. For higher resolution studies, slides were then dipped in Kodak NTB-3 nuclear emulsion and exposed for 3 weeks. An oligonucleotide probe encoding the sense strand (residues 421–468) was used as a negative control. Both antisense oligonucleotide probes gave the same results.

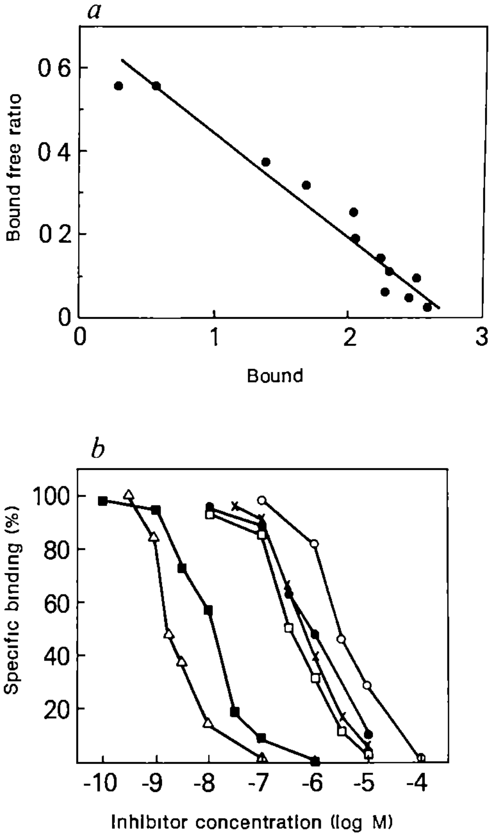


FIG 3 *a*, Scatchard analysis of ^{3}H-labelled kainate binding to transfected COS-7 cell membranes Binding was performed at concentrations of 0 5–100 nM Data were analysed using the LIGAND program[13], and found to fit a single-site model A K_d of 5 6 ± 0 7 nM (mean ± s d , $n = 3$) was obtained Values for the maximum number of binding sites were 2 8 and 3 1 pmol per mg protein for two separate batches of cells Bound are expressed as pmol ^{3}H-labelled kainate per mg protein and bound free ratio is expressed as pmol ^{3}H-labelled kainate per mg protein per nM *b*, Inhibition profiles of various ligands for ^{3}H-labelled kainate binding to the transfected cells Values of 50% inhibitory concentration IC$_{50}$ (mean ± s d , $n = 3$) were 2 7 ± 0 7 nM for domoic acid (△), 13 2 ± 0 5 nM for kainate (■), 0 59 ± 0 35 μM for quisqualate (□), 0 80 ± 0 35 μM for glutamate (●), 0 83 ± 0 13 μM for 6-cyano-7-nitroquinoxaline-2,3-dione (×), and 4 1 ± 1 2 μM dihydrokainate (○)

METHODS An expressible cDNA containing an entire open-reading frame was constructed as follows (see also Fig 1) The cDNA insert of FKA 27C was subcloned into *Eco*RI site of the plasmid bBluescript KS(+) (Stratagene) The *Hin*dIII-*Kpn*I fragment of FKA 17A, the *Kpn*I-blunt-ended *Acc*I fragment of FKA 15C and the *Hin*dIII-*Hin*cII fragment of the plasmid containing FKA 27C cDNA were ligated to yield FKA 271715 The *Bgl*II-*Acc*I fragment of FKA 16A and the *Bam*HI-*Acc*I fragment of the FKA 271715 were ligated to yield FKA 16271715 The *Xba*I-*Apa*I fragment of the plasmid FKA 16271715, containing the expressible cDNA, was subcloned under the SV40 early promoter of a modified form of the plasmid, pcD2 (ref 12) The modified pcD2 plasmid was made by the introduction of multiple cloning sites at *Pst*I and *Bam*HI sites of the original plasmid pcD2 vector The sequence encoding the introduced multiple cloning sites was 5′-TGCAGTCTAGACCCGGGAATTCGGGCCCGGATC-3′ COS-7 cells (5 × 10^5 per 10-cm plate) were cultured overnight and transfected with 20 μg plasmid pcD2 DNA containing the expressible FKA cDNA insert by a calcium phosphate method[12] After 24 h, the medium was changed and plates were incubated for an additional 4 days Cells were collected in distilled water, centrifuged at 54,000*g* for 20 min, resuspended with a polytron in distilled water, and centrifuged at 54,000*g* for 20 min Membrane fractions were frozen as pellets and stored at −70 °C For binding studies, frozen membranes were resuspended with a polytron in 50 mM Tris-HCl, pH 7 0, and centrifuged at 54,000*g* for 20 min The membranes were washed two additional times and subjected to ^{3}H-labelled kainate binding as previously described[3] Untransfected COS-7 cells had no binding activity for ^{3}H-labelled kainate For displacement studies, inhibitors were incubated with membranes for 10 min before addition of ^{3}H-labelled kainate at a final concentration of 5 nM

Northern blot analysis with KBP cDNA probe showed that two principal transcripts of 6 and 4 kilobases were found in frog brain (Fig 2*a*) *In situ* hybridization histochemistry on frog brain sections demonstrated that the pattern of expression of the KBP transcripts was similar to those of the biochemically purified KBP and ^{3}H-labelled kainate-binding sites (Fig 2*b*, and ref 5) The KBP transcripts were abundantly expressed in the torus semicircularis and the optic tectum Higher resolution study revealed that the transcripts were distributed in layers 2, 4 and 6 of the optic tectum Dendrites from neurons in these layers project into layers 3 and 5, where immunostaining was apparent[5]

An expressible cDNA containing the entire open-reading frame was constructed using appropriate restriction-enzyme-digested fragments from the four positive clones in Fig 1*a*, and subcloned under the SV40 early promoter in a modified form of the plasmid pcD2 vector[12] The plasmids were transfected into COS-7 cells by a calcium phosphate method[12] Membrane fractions of the transfected cells were prepared 4 days after transfection and assayed for ^{3}H-labelled kainate-binding activity Scatchard analysis[13] indicated that the membrane fraction of the transfectants contained a single class of ^{3}H-labelled kainate-binding sites with a dissociation constant (K_d) of 5 6 nM (Fig 3*a*) Purified frog brain KBP has both a high ($K_d = 5\ 5$ nM)- and a low ($K_d = 34$ nM)-affinity binding site for ^{3}H-labelled kainate[3] Lack of the low-affinity binding site in the transfectants could indicate the existence of another subunit in the purified frog brain protein Alternatively, different post-translational modifications between frog brain and transfected COS-7 cells could explain the absence Displacement of ^{3}H-labelled kainate binding with selective ligands showed similar patterns to those found in the biochemically purified KBP (Fig 3*b*, and ref 3) Immunoblotting analysis of the membrane preparation using polyclonal antisera against the purified frog KBP revealed that the isolated KBP cDNA produced a protein with a migration rate on SDS-PAGE similar to that of the purified KBP of M_r 48 K (data not shown)

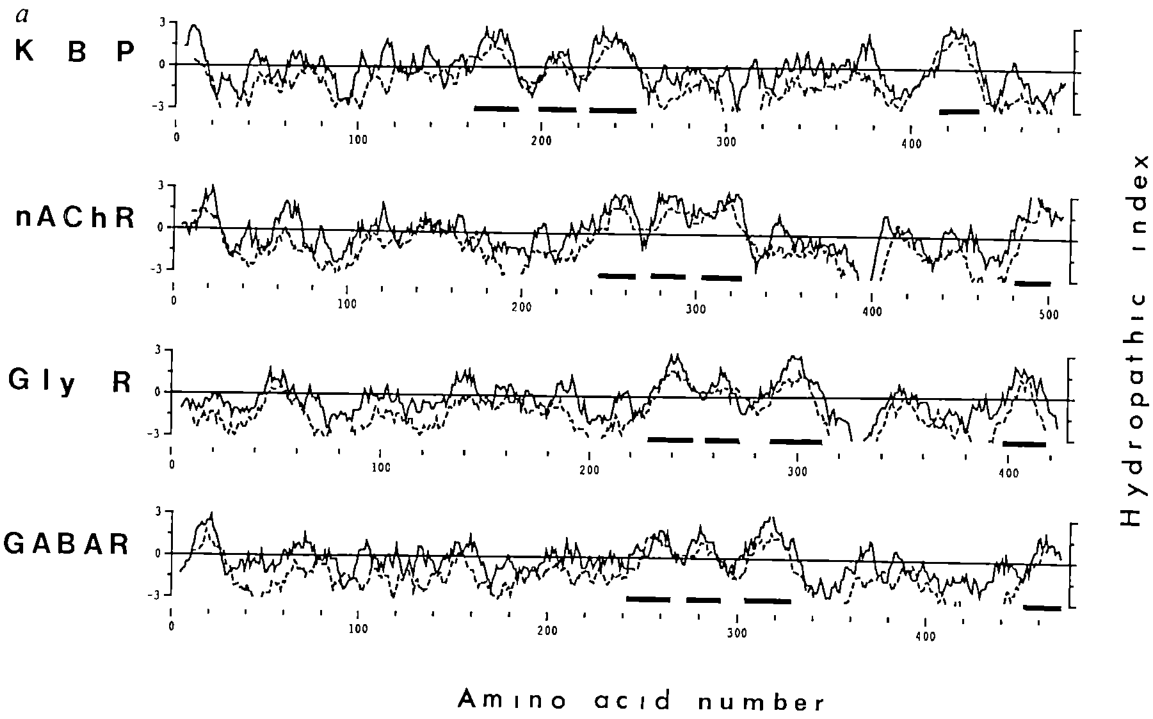
Although the expressed protein in transfected cells could bind kainate, injection of mRNA, transcribed *in vitro* using the bacterial RNA polymerase from the cDNA encoding the entire KBP, did not produce a detectable electrophysiological response to perfused kainate in *Xenopus laevis* oocytes by a two-microelectrode voltage-clamp method In preliminary experiments, co-injection of oligonucleotides complementary to the KBP cDNA sequence with frog brain poly(A)$^+$ RNA[14 15] did not significantly repress an electrophysiological response to kainate compared with control

Analysis for regional hydrophobicity[16,17] of the deduced amino-acid sequence for the KBP revealed four putative transmembrane domains The distribution and spacing of the domains are similar to those seen in various subunits of ligand-gated ion channels, including nicotinic acetylcholine receptors (nAChRs)[7], glycine receptor[14] and γ-aminobutyric-acid receptors[18] (Fig 4*a*) This hydrophobic profile is one of the common structural features presumed to be important for the function of the ligand-gated ion channels[6] But neither the two cysteine residues in the predicted N-terminal extracellular segment nor the proline residue in the first proposed transmembrane domain, which are other important structural features conserved in ligand-gated ion channel subunits[6], is found in the KBP The KBP has a shorter N-terminal and a longer C-terminal hydrophilic segment than do the ligand-gated ion channel subunits

Although homology search in a sequence bank (NBRF) revealed no strong similarities to known proteins, some amino-acid sequence similarity to neuronal nAChR subunits[7] could be found in particular segments, especially in the proposed fourth transmembrane domain (Fig 4*b*) The sequence alignment of the KBP and neuronal nAChR α_2-subunit shows that the serine residue in the proposed second transmembrane domain is conserved (Fig 4b) The equivalent residue in *Torpedo* and muscle nAChR subunits is important for ion transport[19,20] The KBP has negatively charged amino-acid residues around the second putative transmembrane domain Negatively charged amino-acid residues seen in the homologous region of the *Torpedo* nAChR subunits have been proposed to form anionic rings, which are important determinants of the cation transport rates[21] Like the nAChRs, kainate receptors are known to be cation-transfer ion channels[1]

The results presented here show that the KBP cDNA encodes a kainate-binding protein that cannot form a functional channel when expressed alone There are two general interpretations for this finding The first is that the KBP cDNA does not encode a neurotransmitter receptor subunit, but rather an uncharacterized kainate-binding molecule, which may or may not be related to EAA neurotransmission The preliminary results of the complementary oligonucleotide blocking experiments and the lack of certain common structural features in the KBP, which are presumed to be important for channel function and found all ligand-gated ion channel subunits sequenced so far, coul support this interpretation Because KBP is the principal kai ate-binding protein in frog brain[3-5], this interpretation woul imply that most or all of the kainate-binding sites are n associated with a kainate receptor-ionophore complex The fa that the KBP has some structural properties of known ligan gated ion channel subunits indicates that such a binding prote is evolutionarily and functionally related to a recepto ionophore complex

The second interpretation is that the KBP cDNA encodes subunit of a kainate-gated ionophore that requires an addition subunit(s) for physiological activity The requirement of mult ple subunits to produce a functional receptor-ionophore con plex is well documented for nAChRs (refs 7, 22, 23) Injectio of *Torpedo* nAChR α-subunit mRNA alone produces a prote with ligand-binding activity but no channel activity in *Xenop* oocytes[22] Furthermore, the following observations in the prese study support the idea that the KBP is related to a function receptor-ionophore complex (1) COS-7 cells transfected wi the KBP cDNA could bind kainate, (2) hydrophobic profil of the KBP were similar to those of other ligand-gated io channel subunits, (3) some sequence similarities to nACh



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Frog KBP                  ---SKELWIGIIISYVITSLCLFLVGRLSPCEWTEPASEQNQFTLLNSLWYGVGALTLQGAEPQ
                             |     |   |  |       |       |       ||  |         |   |
Rat nAChR α2 Subunit      ---IRRLPLFYTINLIIPCLLISCLTVLVFYL   PSECGEKITLCISVLLSLTVFLLLITEII
                             __________________________          ___________________
                                        TMD 1                           TMD 2

PKALSA    RIIAVIWWVFSITLLAAYIGSFASYINSNTNQ-------TQDQTGWVPVQPHTLGGIFLILGIGLA LGLIVSFMELMCKSRS---
|                  |  | |   |  |                       |  |       ||| | |    || |  |
PSTSLVIPLIGEYLLFTMIF VT LSIVITVFVLNVHHRSPS-------SSVKEDWKYVAM VVDRIFLWLFIIVCFLGTIGLFLPPFLAGMI*
              ________________________                                   ____________________
                       TMD 3                                                    TMD 4
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FIG 4 *a*, Comparison of hydropathy profiles of the KBP α_2-subunit of rat nAChR[7], strychnine-binding subunit of rat glycine receptor (GlyR)[14] and β_1 subunit of bovine γ-aminobutyric acid receptor (GABAR)[18] Scores of hydropathy were computed according to Kyte and Doolittle[16] (solid line) and Engelman, Steitz and Goldman[17] (broken line) with window sizes 9 and 20, respectively Solid bars indicate proposed transmembrane domains

b, Alignment of the amino-acid sequence of the KBP and nAChR α_2 subunit[7] in the putative transmembrane domains Identical residues are indicated by vertical lines Colons indicate conservative substitution of amino-acid residues[27] Gaps of sequence are inserted to achieve maximum similarities Dashes show the sequences not compared TMD, Transmembrane domains subunits were found in certain segments, and (4) there could be an additional homologous subunit(s) forming a functional receptor together with the KBP This possibility is supported by the fact that two bands were detected by northern blotting at high stringency, and that multiple proteins at positions corresponding to a M_r of 48,000 on an SDS-polyacrylamide gel were recognized by immunostaining using polyclonal and monoclonal antibodies against the KBP (refs 3, 4) Furthermore, the KBP cDNA encodes a protein that produces only a high-affinity binding site for kainate in COS-7 cells Although the lack of the previously observed lower-affinity site[3] could be due to different post-translational modifications between frog brain and transfected COS-7 cells, it raises the possibility that a second protein, which contains the low-affinity binding site, is necessary for a physiologically functional receptor

This study provides the first molecular characterization of a component of a kainate receptor-binding system that could be involved in EAA neurotransmission Recent evidence has shown that EAAs are important neurotransmitters in the central nervous system and could be involved in neurotoxicity and several other neurological disorders[1 2] This provides an impetus to characterize their receptors and related proteins Further studies focused on isolating and expressing additional subunits of KBP should help elucidate the precise role of the protein and its relationship to neurotransmission and kainate neurotoxicity ☐

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1 Monaghan D T Bridge R J & Cotman C W *A Rev Pharmac Tox* **29**, 365-402 (1989)
2 Foster A C & Fagg G *Brain Res Rev* **7**, 103-164 (1984)
3 Hampson D R & Wenthold R J *J biol Chem* **263**, 2500-2505 (1988)
4 Hampson D R Wheaton K D Dechesne C J & Wenthold R J *J biol Chem* **264**, 13329-13335 (1989)
5 Dechesne C J *et al J Neurosci* (in the press)
6 Barnard E A Darlison M G & Seeburg P *Trends Neurosci* **10**, 502-509 (1987)
7 Wada K *et al Science* **240**, 330-334 (1988)
8 Saiki R K *et al Science* **239**, 487-491 (1988)
9 Kozak M *Nucleic Acids Res* **12**, 857-872 (1984)
10 von Heijne G *Nucleic Acids Res* **14**, 4683-4690 (1986)
11 Marshall R D *Biochem Soc Symp* **40**, 17-26 (1974)
12 Chen C & Okayama H *Molec cell Biol* **7**, 2745-2752 (1987)
13 Munson P J & Rodbard D *Analyt Biochem* **98**, 39-45 (1980)
14 Grenningloh G *et al Nature* **328**, 215-220 (1987)
15 Sumikawa K & Miledi R *Proc natn Acad Sci USA* **85**, 1302-1306 (1988)
16 Kyte J & Doolittle R F *J molec Biol* **157**, 105-132 (1982)
17 Engelman D M Steitz T A & Goldman A *A Rev Biophys biophys Chem* **15**, 321-353 (1986)
18 Schofield P R *et al Nature* **328**, 221-227 (1987)
19 Giraudat J *et al Biochemistry* **26**, 2410-2418 (1987)
20 Leonard R J Labarca C G Charnet P Davidson N & Lester H A *Science* **242**, 1578-1581 (1988)
21 Imoto K *et al Nature* **335**, 645-648 (1988)
22 Kurosaki T *et al FEBS Lett* **214**, 253-258 (1987)
23 Boulter J *et al Proc natn Acad Sci USA* **84**, 7763-7767 (1987)
24 Maniatis T Fritsch E F & Sambrook J *Molecular Cloning A Laboratory Manual* (Cold Spring Harbor Laboratory New York 1982)
25 Sanger F Nicklen S & Coulson A R *Proc natn Acad Sci USA* **74**, 5463-5467 (1977)
26 Okayama H *et al Meth Enzym* **154**, 3-28 (1987)
27 Dayhoff M O Schwartz R M & Orcutt B C in *Atlas of Protein Sequence and Structure* Vol 5 suppl 3 (ed Dayhoff M O) 345-352 (National Biomedical Research Foundation Silver Spring Maryland 1978)

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Molecular structure of the chick cerebellar kainate-binding subunit of a putative glutamate receptor

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KAINATE receptors mediate some of the excitatory transactions carried out in the central nervous system by the neurotransmitter glutamate[1] They are involved in neurotoxicity[2], possibly in neurodegenerative disorders[3] and it has been suggested that they have a role in long-term potentiation[4] Kainate receptors are present both on neuronal[1] and glial[5–7] cell membranes where they regulate the gating of a voltage-independent ion channel[8] Nothing is known about their molecular structure Taking advantage of the unusually high abundance of ^{3}H-kainate binding sites in the chick cerebellum[9,10], we have isolated an oligomeric protein that displays a pharmacological profile similar to that of a kainate receptor, and have demonstrated, using the monoclonal antibody IX-50, that this protein is composed of a single polypeptide of M_r 49,000 which harbours the specific kainate recognition site[11] The structure of this kainate binding protein (KBP) is also of interest because of its exclusive cerebellar localization on Bergmann glial membrane[12] in close proximity to established glutamatergic synapses[13] We now report the isolation of the complementary DNA containing the complete coding region of the kainate binding protein The predicted structure of the mature protein has four putative transmembrane domains with a topology analogous to that found in the superfamily of ligand-gated ion channels[14–16] This raises the possibility, that kainate binding protein may form part of an ion channel and may be a subunit of a kainate subtype of glutamate receptor

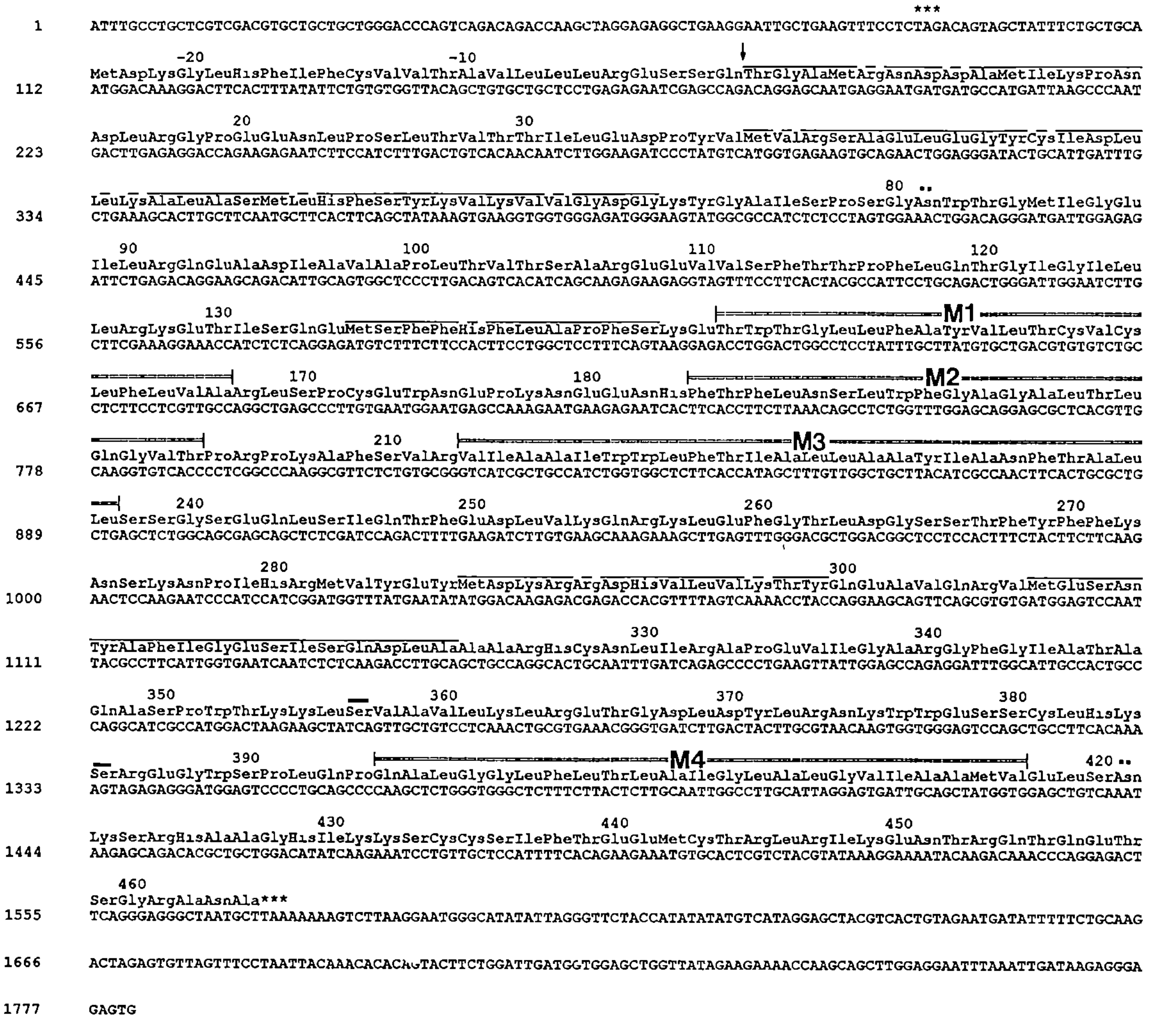
The chick cerebellar kainate binding protein (KBP) was isolated as previously described[11], as a single polypeptide of M_r 49K with a purity, estimated on SDS-polyacrylamide gels, of 80–95% KBPs with similar M_r values have been isolated from frog[17] and pigeon[18] brain Microsequencing of the N terminus of purified KBP produced only one sequence To obtain internal amino-acid sequences of KBP, the latter was fragmented with cyanogen bromide and the five most prominent peptides (I–V), obtained on separation by reverse-phase HPLC, were submitted to N-terminal sequence analysis (P G and V I T, manuscript in preparation) All sequences determined chemically are shown (solid lines) in Fig 1

As peptide IV was immunopositive with monoclonal antibody IX-50 and polyclonal antibodies (data not shown) we synthesized a set of 20-nucleotide-long oligodeoxyribonucleotides (oligo(dT)) corresponding to its N terminus (probe IV, see Fig 2 legend) To establish its suitability for screening libraries and the optimal hybridization conditions, probe IV was used to analyse RNA blots As shown in Fig 2*a* (panel A), probe IV hybridized mainly to a 3 9-kilobase (kb) RNA, but also to a 6-kb RNA Both of these are much more abundant in the cerebellum than in the cerebrum and not detectable in the liver This tissue distribution correlates very well with that of kainate-binding sites[19] and with anti-KBP immunoreactivity (data not shown) Analysis of RNA blots with probe I, derived from the sequence of peptide I, gives an identical pattern to the one shown in Fig 2*a*, panel A

An oligo(dT)-primed chick cerebellar cDNA library, constructed in phage λgt-10, was screened with the probe IV (for methods, see Fig 2 legend) Positive phages were rescreened with probe I and cDNAs KBP-2 9 and KBP-2 2 were isolated Radiolabelled KBP-2 2 cross-hybridizes to KBP-2 9 and gives the anticipated hybridization pattern on northern blots (Fig 2*a*, panel B) Rescreening the same library with restriction fragments of cDNA KBP-2 2 resulted in isolation of the clone KBP-3 3, which has the complete coding region of KBP The maps of clones used to establish the coding DNA sequence of KBP are shown in Fig 2*b*

The entire protein-coding DNA sequence and deduced amino-acid sequence of KBP are shown in Fig 1 The mature protein is predicted to have 464 amino acids and an M_r of 51 8K The 23 residues preceding the N-terminal sequence probably constitute the signal peptide The first methionine is preceded by an in-frame stop codon and has a sequence context consistent with its use as a translation start site[20] Although we have not formally shown that the cloned cDNA expresses a protein with kainate-binding properties, several arguments indicate that the derived amino-acid sequence corresponds to that of KBP First, the deduced amino-acid sequence contains all the peptide sequences derived from the pure 49K polypeptide harbouring the kainate-binding site[11] Second, two of the encoded peptides (IV and V) provide determinants for polyclonal antibodies directed against KBP and peptide IV is recognized by the anti-KBP monoclonal antibody IX-50 Third, the amino-acid composition and size of the deduced sequence are, within limits of error, consistent with those of isolated KBP Finally, the preferential cerebellar localization of the RNA species hybridizing to the cloned cDNA is strictly in line with the selective tissue distribution of KBP

Data-base searches did not reveal any significant sequence homology of KBP to other proteins A comparison of the overall pattern of hydrophobic residues for KBP with such patterns for known ligand-gated channels (Fig 3), however, revealed some similarities The KBP contains several hydrophobic regions including prominent ones near the N and C termini of the protein In addition, there are three stretches of hydrophobicity between residues 140 and 240, each of which is long enough to span a lipid bilayer in an α-helical configuration One interpretation of these data is that the first hydrophobic region functions as a signal peptide and that the other four regions (M1-M4) serve as membrane spanning regions The size of KBP and the overall placement of the putative membrane spanning regions of KBP are similar to the size of known ligand-gated ion channel proteins and to the placement of putative membrane spanning regions of these proteins indicating that KBP has a general architecture similar to that of the chemically gated ion channels If KBP resembles ligand-gated ion channels, the N and C termini are probably located extracellularly This assignment is supported by the presence of putative N-glycosylation sites at positions 81 and 421, in the putative N- and C-terminal extracellular regions, and by the presence of putative phosphorylation sites



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                                                                                                   ***
1     ATTTGCCTGCTCGTCGACGTGCTGCTGCTGGGACCCAGTCAGACAGACCAAGCTAGGAGAGGCTGAAGGAATTGCTGAAGTTTCCTCTAGACAGTAGCTATTTCTGCTGCA

           -20                 -10                                 ↓
      MetAspLysGlyLeuHisPheIlePheCysValValThrAlaValLeuLeuLeuArgGluSerSerGlnThrGlyAlaMetArgAsnAspAspAlaMetIleLysProAsn
112   ATGGACAAAGGACTTCACTTTATATTCTGTGTGGTTACAGCTGTGCTGCTCCTGAGAGAATCGAGCCAGACAGGAGCAATGAGGAATGATGATGCCATGATTAAGCCCAAT

                20                            30
      AspLeuArgGlyProGluGluAsnLeuProSerLeuThrValThrThrIleLeuGluAspProTyrValMetValArgSerAlaGluLeuGluGlyTyrCysIleAspLeu
223   GACTTGAGAGGACCAGAAGAGAATCTTCCATCTTTGACTGTCACAACAATCTTGGAAGATCCCTATGTCATGGTGAGAAGTGCAGAACTGGAGGGATACTGCATTGATTTG

                                                                                                80 ••
      LeuLysAlaLeuAlaSerMetLeuHisPheSerTyrLysValLysValValGlyAspGlyLysTyrGlyAlaIleSerProSerGlyAsnTrpThrGlyMetIleGlyGlu
334   CTGAAAGCACTTGCTTCAATGCTTCACTTCAGCTATAAAGTGAAGGTGGTGGGAGATGGGAAGTATGGCGCCATCTCTCCTAGTGGAAACTGGACAGGGATGATTGGAGAG

         90                            100                           110                           120
      IleLeuArgGlnGluAlaAspIleAlaValAlaProLeuThrValThrSerAlaArgGluGluValValSerPheThrThrProPheLeuGlnThrGlyIleGlyIleLeu
445   ATTCTGAGACAGGAAGCAGACATTGCAGTGGCTCCCTTGACAGTCACATCAGCAAGAGAAGAGGTAGTTTCCTTCACTACGCCATTCCTGCAGACTGGGATTGGAATCTTG

                130                                                              M1
      LeuArgLysGluThrIleSerGlnGluMetSerPhePheHisPheLeuAlaProPheSerLysGluThrTrpThrGlyLeuLeuPheAlaTyrValLeuThrCysValCys
556   CTTCGAAAGGAAACCATCTCTCAGGAGATGTCTTTCTTCCACTTCCTGGCTCCTTTCAGTAAGGAGACCTGGACTGGCCTCCTATTTGCTTATGTGCTGACGTGTGTCTGC

                            170                           180                              M2
      LeuPheLeuValAlaArgLeuSerProCysGluTrpAsnGluProLysAsnGluGluAsnHisPheThrPheLeuAsnSerLeuTrpPheGlyAlaGlyAlaLeuThrLeu
667   CTCTTCCTCGTTGCCAGGCTGAGCCCTTGTGAATGGAATGAGCCAAAGAATGAAGAGAATCACTTCACCTTCTTAAACAGCCTCTGGTTTGGAGCAGGAGCGCTCACGTTG

                                   210                                     M3
      GlnGlyValThrProArgProLysAlaPheSerValArgValIleAlaAlaIleTrpTrpLeuPheThrIleAlaLeuLeuAlaAlaTyrIleAlaAsnPheThrAlaLeu
778   CAAGGTGTCACCCCTCGGCCCAAGGCGTTCTCTGTGCGGGTCATCGCTGCCATCTGGTGGCTCTTCACCATAGCTTTGTTGGCTGCTTACATCGCCAACTTCACTGCGCTG

               240                           250                           260                           270
      LeuSerSerGlySerGluGlnLeuSerIleGlnThrPheGluAspLeuValLysGlnArgLysLeuGluPheGlyThrLeuAspGlySerSerThrPheTyrPhePheLys
889   CTGAGCTCTGGCAGCGAGCAGCTCTCGATCCAGACTTTTGAAGATCTTGTGAAGCAAAGAAAGCTTGAGTTTGGGACGCTGGACGGCTCCTCCACTTTCTACTTCTTCAAG

                     280                                                              300
      AsnSerLysAsnProIleHisArgMetValTyrGluTyrMetAspLysArgArgAspHisValLeuValLysThrTyrGlnGluAlaValGlnArgValMetGluSerAsn
1000  AACTCCAAGAATCCCATCCATCGGATGGTTTATGAATATATGGACAAGAGACGAGACCACGTTTTAGTCAAAACCTACCAGGAAGCAGTTCAGCGTGTGATGGAGTCCAAT

                                                                          330                           340
      TyrAlaPheIleGlyGluSerIleSerGlnAspLeuAlaAlaAlaArgHisCysAsnLeuIleArgAlaProGluValIleGlyAlaArgGlyPheGlyIleAlaThrAla
1111  TACGCCTTCATTGGTGAATCAATCTCTCAAGACCTTGCAGCTGCCAGGCACTGCAATTTGATCAGAGCCCCTGAAGTTATTGGAGCCAGAGGATTTGGCATTGCCACTGCC

           350                           360                           370                           380
      GlnAlaSerProTrpThrLysLysLeuSerValAlaValLeuLysLeuArgGluThrGlyAspLeuAspTyrLeuArgAsnLysTrpTrpGluSerSerCysLeuHisLys
1222  CAGGCATCGCCATGGACTAAGAAGCTATCAGTTGCTGTCCTCAAACTGCGTGAAACGGGTGATCTTGACTACTTGCGTAACAAGTGGTGGGAGTCCAGCTGCCTTCACAAA

                    390                                               M4                                     420 ••
      SerArgGluGlyTrpSerProLeuGlnProGlnAlaLeuGlyGlyLeuPheLeuThrLeuAlaIleGlyLeuAlaLeuGlyValIleAlaAlaMetValGluLeuSerAsn
1333  AGTAGAGAGGGATGGAGTCCCCTGCAGCCCCAAGCTCTGGGTGGGCTCTTTCTTACTCTTGCAATTGGCCTTGCATTAGGAGTGATTGCAGCTATGGTGGAGCTGTCAAAT

                               430                           440                           450
      LysSerArgHisAlaAlaGlyHisIleLysLysSerCysCysSerIlePheThrGluGluMetCysThrArgLeuArgIleLysGluAsnThrArgGlnThrGlnGluThr
1444  AAGAGCAGACACGCTGCTGGACATATCAAGAAATCCTGTTGCTCCATTTTCACAGAAGAAATGTGCACTCGTCTACGTATAAAGGAAAATACAAGACAAACCCAGGAGACT

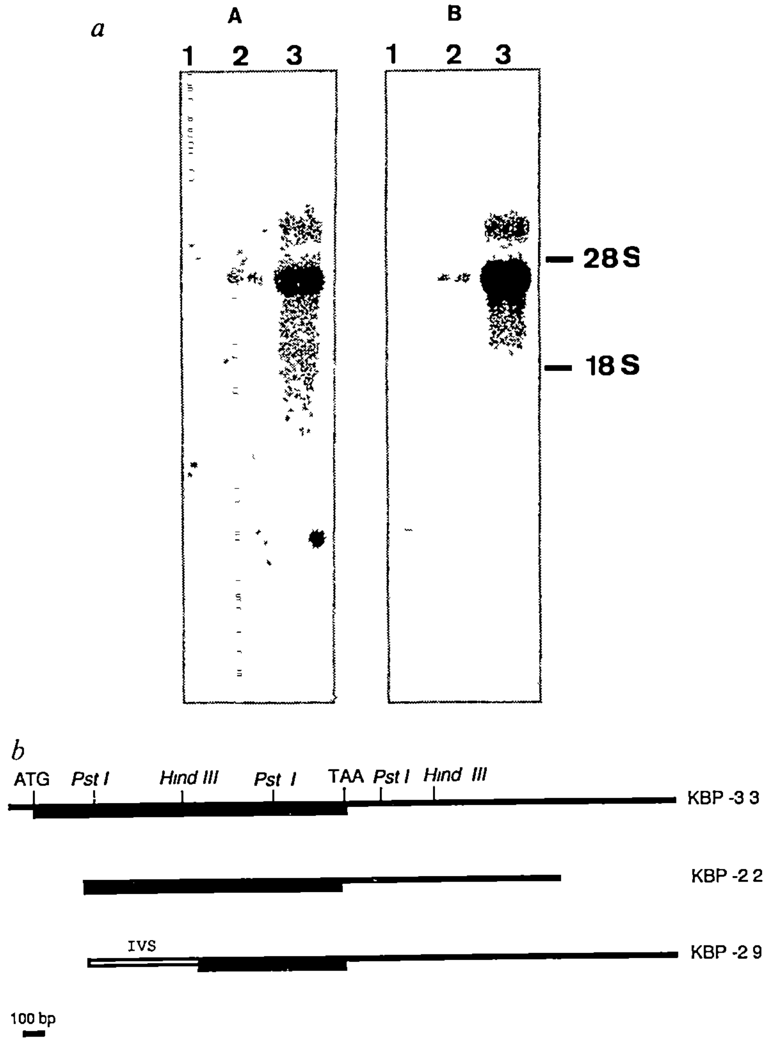
         460
      SerGlyArgAlaAsnAla***
1555  TCAGGGAGGGCTAATGCTTAAAAAAAGTCTTAAGGAATGGGCATATATTAGGGTTCTACCATATATATGTCATAGGAGCTACGTCACTGTAGAATGATATTTTTCTGCAAG

1666  ACTAGAGTGTTAGTTTCCTAATTACAAACACACAGTACTTCTGGATTGATGGTGGAGCTGGTTATAGAAGAAAACCAAGCAGCTTGGAGGAATTTAAATTGATAAGAGGGA

1777  GAGTG
```

FIG 1 DNA sequence and deduced amino-acid sequence of the chick cerebellar kainate binding protein. Nucleotides are numbered at left and amino acids above the sequence The first in-frame stop codon in the 5′ untranslated sequence and the stop codon at the end of the coding region are labelled with asterisks Amino-acid numbering is from the first residue of the mature N-terminal sequence as established by direct N-terminal sequencing of the kainate binding protein The presumptive signal peptide sequence is indicated by negative numbering Peptide sequences determined chemically are denoted by solid lines and uncertain or unidentified residues by broken lines Signal peptide cleavage site is indicated by arrow The proposed membrane-spanning hydrophobic sequences are indicated by double lines (M1-M4) The putative extracellular N-glycosylation sites are labelled with two dots and the putative intracellular phosphorylation sites by a solid bar

METHODS The cDNA inserts of positive phages were subcloned into Bluescript phagemid pKS vectors (Stratagene) and sequenced by the dideoxy chain termination method[30] The sequence presented is combined from all cDNAs and both strands were analysed The 3′ untranslated regions of the cDNA clones beyond the *Pst*I site were not sequenced completely on both strands and are not shown



IG 2 Molecular cloning of the KBP cDNA *a*, Northern blot nalysis of chick RNA with oligodeoxyribonucleotide oligo-(dT) robe IV corresponding to peptide IV (panel A) and with cloned BP-2 2 cDNA (panel B) Line 1, liver, line 2, cerebrum, line 3, erebellum *b*, cDNA clones and partial restriction map All DNAs have their cognate 3′ poly-A sequences The 3′ ntranslated region of KBP-2 2 beyond the *Hind* III site is ifferent from KBP-3 3 and KBP-2 9, probably as a result of lternate 3′-end processing The open reading frame of KBP-9 was truncated at its 5′ side, probably as a result of complete splicing Solid box, protein coding region, IVS, resumed intervening sequence

/IETHODS Standard recombinant DNA methods were as lescribed[25] Based on the sequence of peptide IV, the oligo-dT) 5′-AARGCGTAGTTGSWCTCCAT-3′ (where R=A or G, S=C r G, W=A or T)[26] was synthesized (probe IV) and labelled o high specific radioactivity with [γ-^{32}P]ATP[27] Total RNA was solated[28] from adult chick tissues, 25 μg per line was electrophoresed on a 1% formaldehyde agarose gel, blotted on itrocellulose membrane (Hybond-C, Amersham) and hybridized overnight with the radiolabelled oligo-(dT) probe at 37 °C n 5×SSPE (1×SSPE contained 0 18 M NaCl, 0 01 M sodium hosphate, 1 mM EDTA, pH 6 8) as described[27] The high stringency wash was at 37 °C in 0 5×SSPE for 1 h Antisense robe I based on peptide I sequence was synthetized as an oligonucleotide mixture 5′-TAIGTIACIACIAGIACRTGRTCICAIC-QCTTRTTC-3′ (where I=inosine, Q=G or T and R=A or G), nd radiolabelled as above To isolate KBP cDNAs, an oligo-dT)-primed cDNA library in phage λgt-10 (ref 29) was constructed from cerebellar poly(A)$^+$-RNA of hatching chicks Double-stranded cDNA was synthesized, fitted with *Eco*RI inkers, and cDNAs were size fractionated on 1% agarose gel Fragments larger than 1 5 kb were isolated and ligated with *Eco*RI cut λgt-10, resulting in 150,000 independent ecombinants of which at least 50% carried inserts The ibrary was amplified and ~160,000 phages screened with robe IV as described above, positives were rescreened with robe I resulting in isolation of KBP-2 9 and KBP-2 2 clones A screen of 300,000 phages, under high stringency conditions, with the 0 8 kb *Pst*I-*Pst*I and 0 1 kb *Eco*RI-*Pst*I fragments derived from the 5′ end of KBP-2 2, led to the isolation of the clone KBP-3 3

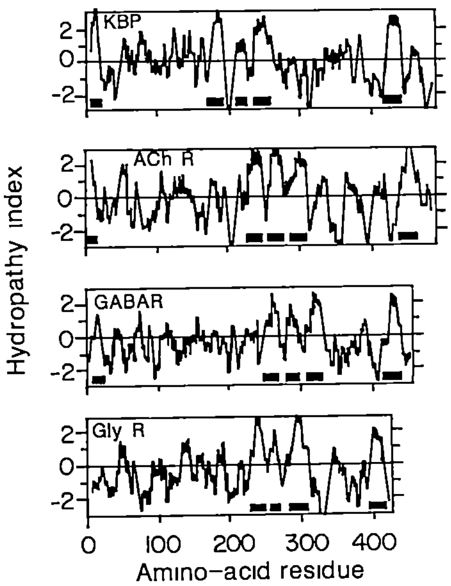


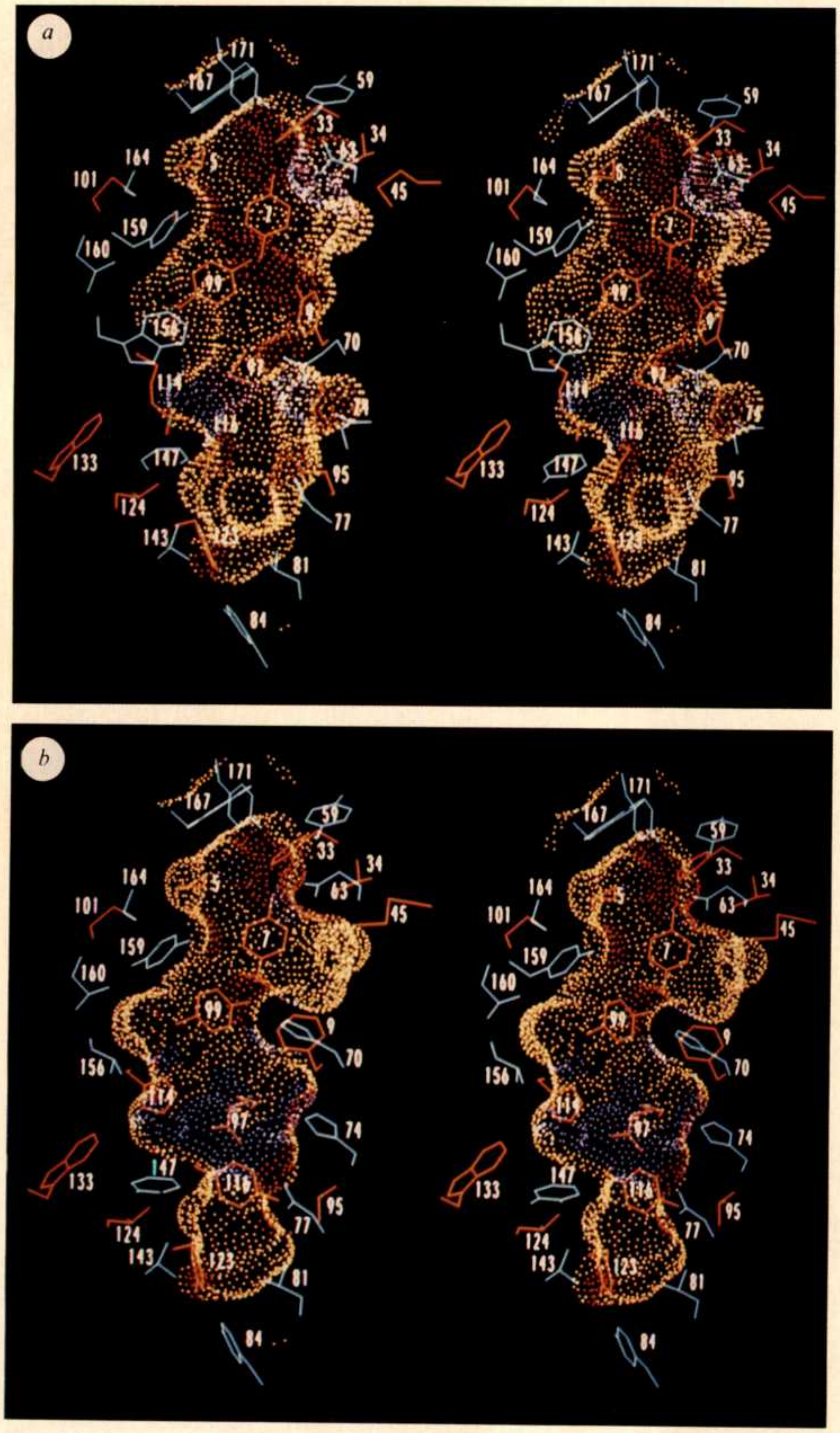
FIG 3 Structural similarity of the KBP to subunits of ligand-gated ion channels Hydropathy profiles computed according to Kyte and Doolittle[31], averaged over a window of 9 residues and plotted with a 1-residue interval Solid box, putative signal peptide and membrane spanning regions AChR, α2 chick acetylcholine receptor subunit[32], GABA R, γ-amino-butyric acid receptor α subunit[15], Gly R, glycine receptor[16]

at positions 357 and 385, in the putative intracellular region between M3 and M4

Further analysis of KBP structure shows the presence of features compatible with channel function, particularly in its putative M2 transmembrane segment Indeed, the M2 domain of the various nicotinic acetylcholine receptor (AChR) subunits[14], which is essential to channel function[21-23], contains polar uncharged residues and is preceded by hydrophilic negatively charged residues Similar features in the equivalent region of KBP are observed, indicating, only by analogy, a possible role in ion permeation As mentioned above, KBP also contains potential phosphorylation sites, a finding consistent with the reported modulation of kainate-gated currents by protein kinase[24] If indeed, KBP forms part of an ion channel, its sequence does not display the homologies that have been used to group the various chemically gated ion channels in a single family The structure of KBP must therefore reflect its idiosyncratic properties, one of which is its unusually high density in the cerebellum and exclusive presence on Bergmann glia (ref 12 and P Somogyi, N Eshhar, V I T & J D Roberts, unpublished observations) Although its function is not yet known, its localization on Bergmann glial membranes surrounding the parallel fibre—Purkinje cell glutamatergic synapse indicates a role in modulation of synaptic efficacy Of relevance are our recent findings that isolated Bergmann glial cells in culture display high levels of KBP, depolarize in response to kainate and release GABA (γ-aminobutyric acid) (ref 12 and A Ortega and V I T, unpublished observations) The structural data presented here, together with the above observations, indicate therefore that KBP may be a subunit of a glial subtype of kainate receptor/cation channel ☐

provides the basis for allele-specific nonresponsiveness to certain immunological challenges[10,11,15,16]. Here we report the structure of HLA-Aw68 refined to a resolution of 2.6 Å, which when compared with the 2.7 Å resolution structure of HLA-A2 (M.A.S. *et al.*, manuscript in preparation, and ref. 17) suggests mechanisms for both the broad specificity and the allelic selectivity of peptide binding.

The structure of papain-solubilized HLA-Aw68 was determined and refined to 2.6-Å resolution (see ref. 18 for details) from orthorhombic crystals isomorphous to HLA-A2 crystals[19]. The overall structure, excluding the 13 amino-acid differences, is very similar to that of HLA-A2 refined to high resolution from both monoclinic crystals (2.7 Å; M.A.S. *et al.*, manuscript in preparation) and orthorhombic (2.5 Å) crystals[17]. Figure 1b shows the α-carbon backbone of HLA-Aw68 overlaid on the coordinates of the 2.7 Å resolution monoclinic crystal structure of HLA-A2 and lists root mean square (r.m.s.) deviations in coordinates between domains of the two structures. The average deviations are within the expected experimental error in the coordinates of these structures (Fig. 1*b*, legend).



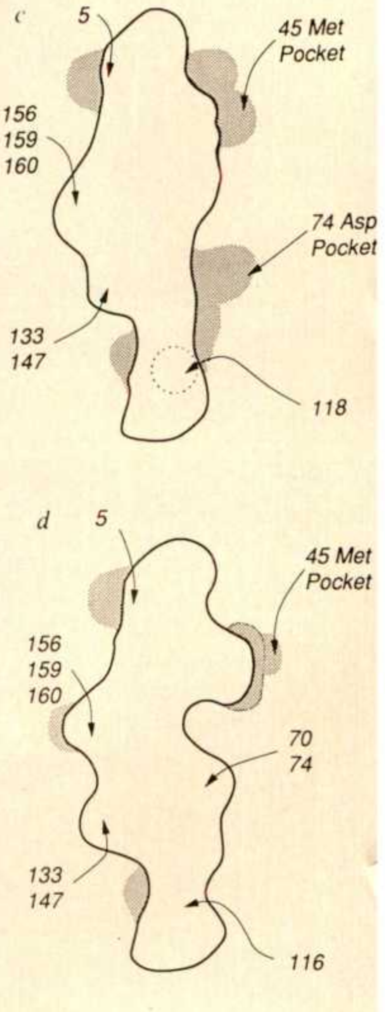


FIG. 3 Top view, comparison of binding site of HLA-Aw68 with HLA-A2. Stereograms show solvent contact surfaces of the clefts coloured by side-chain atom type: red, oxygen; blue, nitrogen; yellow, carbon; purple, sulphur. Side chain colours indicate secondary structure: red side chains, β-sheet; blue side chains, α-helix. *a*, HLA-Aw68 shows prominent new pockets near positions 74 and 116 and a ridge at positions 156 and 70. *b*, HLA-A2 shows Arg 97, Tyr 116, and His 74 filling the pockets. Both clefts have a pocket at 45. *c*, *d* Schematic outline of HLA-Aw68 (*c*) and HLA-A2 (*d*) cleft with pockets labelled. *a*, *b*, Constructed with HYDRA (R. Hubbard, unpublished) and surfaces were calculated using the program MS (ref. 29). Single-letter amino-acid code.

be present but that the Asp 74 pocket would probably be blocked by Arg 97 and Tyr 116 (as in HLA-A2), possibly explaining why one HLA-A2-specific peptide antigen also interacts with HLA-Aw69 (ref. 23).

Comparison of the three-dimensional structures of HLA-Aw68 and HLA-A2 demonstrates how different pockets or subsites within the cleft of the class I histocompatibility glycoproteins are produced by substitutions at positions of amino-acid polymorphism (compare Fig. 3*c* and *d*). The difference between these two allele products is typical of MHC allelic diversity, and so the same degree of structural change in pockets and subsites should be observed for other alleles. In the case we have described, polymorphism results in limited local structural changes; the backbone structure is generally not altered (Fig. 1*b*), and residues identical in both structures remain essentially unperturbed[18]. The observed effects of polymorphism were on the shape (for example, intrusions and ridges) an charge-distribution of the antigen-binding site, and on som local pockets, resulting in changes in their specificity and i their distribution in the site. These structural changes provid the basis for allelic specificity in peptide affinity[11,15,16] and th resultant non-responsiveness (and possibly hyper-responsiveness) to certain immunological challenges[10]. Because amino acid side chains from antigenic peptides could bind in thes pockets, peptides binding to one MHC allelic product coul then have a particular pattern of side chains[24,25] to fit the pockets Moreover, because the affinity of antigenic peptides fo individual histocompatibility glycoproteins may depend on onl a few of an antigenic peptide's side-chains fitting into a subse of the available pockets[26,27], a broad range of peptides coul be accommodated by the various combinations of filled an empty pockets. □

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1. Bjorkman, P. J. *et al. Nature* **329,** 506–512 (1987).
2. Bjorkman, P. J. *et al. Nature* **329,** 512–518 (1987).
3. Holmes, N. & Parham, P. *EMBO J.* **4,** 2849–2854 (1985).
4. Koller, B. H. & Orr, H. T. *J. Immun.* **134,** 2727–2733 (1985).
5. Townsend, A. R. M., Gotch, F. M. and Davey, J. (1985) *Cell* **42,** 457 (1985).
6. Germain, R. M. *Nature* **322,** 687 (1986).
7. Bevan, M. *Nature* **325,** 192 (1987).
8. Townsend, A. & Bodmer, H. *A. Rev. Immun.* **7,** 601–624 (1989).
9. Parham, P. *et al. Proc. natn. Acad. Sci. U.S.A.* **85,** 4005–4009 (1988).
10. Babbitt, B., Allen, P., Matsueda, E., Haber, E. & Unanue, E. *Nature* **317,** 359–361 (1985).
11. Buus, S., Sette, A., Colon, S. M., Miles, C & Grey, H. M. *Science* **235,** 1353–1358 (1987).
12. Sette, A., Buus, S., Colon, S. M., Miles, C. & Grey, H. M. *J. Immun.* **141,** 45–48 (1988).
13. Townsend, A. R. M. *et al., Cell* **44,** 959–968 (1986).
14. Maryanski, J. L., Pala, P., Coradin, J., Jordan, B. B. & Cerotini, J. C. *Nature* **324,** 578–579 (1986).
15. McDevitt, H. O. & Tyan, M. L. *J. expl. Med.* **128,** 1–11 (1968).
16. Fox, B. S., Carbone, F. R., Germain, R. N., Paterson, Y. & Schwartz, R. H. *Nature* **331,** 538–540 (1988).
17. Madden, D. R. *et al. Cold Spring Harb. Symp. Quant. Biol.* **54,** (in the press).
18. Garrett, T. P. J., Saper, M. A. & Wiley, D. C. *J. molec. Biol.* (submitted).
19. Bjorkman, P. J., Strominger, J. L. & Wiley, D. C. *J. molec. Biol.* **186,** 205–210 (1985).
20. Salter, R. D. *et al. Nature* **338,** 345–347 (1989).
21. Connolly, J. M., Potter, T. A., Wormstall, E-M. & Hansen, T. H. *J. exp. Med.* **168,** 325–341 (1988).
22. Taurog, J. D. & El-Zaatari, A. K. *J. clin. Invest.* **82,** 987–992 (1988).
23. Bodmer, H. C., Gotch, F. M. & McMichael, A. J. *Nature* **337,** 653–655 (1989).
24. Sette, A. *et al. Proc. natn. Acad. Sci. U.S.A.* **86,** 3296–3330 (1989).
25. Rothbard, J. B. & Taylor, W. R. *EMBO J.* **7,** 93–100 (1988).
26. Jardetzky, T. S. *et al. EMBO J.* (submitted).
27. Maryanski, J. L., Verdini, A. S., Weber, P. C., Salemme, F. R. & Corradin, G. *Cell* (submitted).
28. Ellis, S. A., Strachan, T., Palmer, M. S. & McMichael, A. J. *J. Immun.* **142,** 3281–3285 (1989).
29. Connolly, M. L. *Quantum Chemistry Program Exchange Bull.* **1,** 74.
30. Brünger, A. T., Kuriyan, J. & Karplus, M. *Science* **235,** 458–460 (1987).
31. Brünger, A. T. in *Topics in Molecular Biology* (ed. Goodfellow, J. M.) (in the press).

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Limited receptor repertoire in a mycobacteria-reactive subset of $\gamma\delta$ T lymphocytes

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THE physiological role of lymphocytes bearing the $\gamma\delta$ T-cell receptor (TCR) is still unclear. A function for a subset of these cells, however, is inferred from the finding that certain $\gamma\delta$ chain-bearing lymphocytes are stimulated in a receptor-dependent fashion by mycobacterial antigens[1–5]. We found that hybridomas derived from such cells in newborn murine thymus not only responded to mycobacterial purified protein derivative (PPD), but also exhibited an apparent autoreactivity[1]. In neither response was haplotype-specific major histocompatibility (MHC) restriction demonstrable. To investigate the nature of antigen recognition by these $\gamma\delta^+$ cells, we sequenced the γ- and δ-chains from 28 PPD-reactive hybridomas, and found that a specific γ-chain, together with one of a limited set of δ-chains, was needed to generate the PPD specificity. The reactive $\gamma\delta$ pairs exhibited considerable junctional diversity, which may act to produce differences in the fine specificities of the responding cells.

The TCR proteins from several PPD-reactive $\gamma\delta$ T-cell hybridomas[1] were compared with those from a collection of non-PPD-reactive $\gamma\delta$-hybridomas[1] by SDS-PAGE (Fig. 1, and data not shown). Most of the PPD-reactive hybridomas shared a δ-chain (the upper band of the receptor doublet) that was not frequently found among the nonreactive population (see also ref. 1). Although γ-chains showed a wider variability in relative molecular mass (M_r) than did δ-chains, all of the PPD-reactive and two of the nonreactive hybridomas possessed a γ-chain of the same M_r (39,000; the lower band of the receptor doublet). The size of this γ-chain indicated that it contained Cγ4 segment (ref. 6). Using a $V\gamma 1$-$C\gamma 4$ combination of oligonucleotide primers, polymerase chain reaction (PCR) amplification products were generated from the messenger RNA of all 28 of the PPD-reactive hybridomas and from 7 of the 25 non-PPD-reactive hybridomas. Sequence analysis revealed an in-frame $V\gamma 1JC\gamma 4$ transcript[7] (Fig. 2*a*) in all of the PPD-reactive hybridomas and only two of the non-PPD-reactive hybridomas. There did not seem to be selection for a particular *VJ* junctional sequence among the PPD-reactive hybridomas (Fig. 2*b*).

Based on our previous analysis, δ-chain gene oligonucleotides were designed that would amplify the $V\delta 6.1$ gene segment ($V\delta m23$ in refs 1 and 8), as well as the closely related $V\alpha 7$ and $V\delta 6$ gene family members[8–12]. Amplification products were generated with these oligonucleotides from 25 of the PPD-reactive and 3 of the non-PPD-reactive hybridomas. Sequence analysis revealed the use of two different *V* region gene segments (Fig. 2*a*). One was identical to the previously described $V\alpha 7.1$ (ref. 10). We have designated this $V\delta$ gene segment $V\delta 6.3$. The partial sequence obtained from the other *V* gene segment was identical to that previously described for the plasmid pλ12 member of the $V\delta 6$ family[13]. The predicted junctional amino acids for all of the $V\delta 6$ chains are listed in Fig. 2*b*, with the three non-PPD-reactive hybridomas indicated by asterisks. All of the $V\delta 6.3$ and $V\delta 6$ ($p\lambda 12$)-expressing hybridomas used the $D\delta 2$ and $J\delta 1$ gene segments, as do most $\gamma\delta$ T cells[8,11,14,15]. Characteristic of adult (as opposed to fetal) $\gamma\delta$ cells[11,14], $D\delta 1$ sequences were present in two of the hybridomas (BNT-9.12 and BNT-21.32), each in a different reading frame. This, and the presence of substantial

N region diversity in most of the PPD-reactive hybridomas, indicate that these cells appear relatively late in ontogeny[11]. All three different reading frames of the *Dδ2* gene segment could be used with retention of PPD specificity. Also, expression of different *N* region-encoded amino acids did not abolish the reactivity.

In both the δ- and the γ-chain, there were no apparent differences between the junctional regions in PPD-reactive versus non-PPD-reactive hybridomas that could account for the difference in reactivity. Instead, PPD-reactivity seems to result from the pairing of a Vγ1JCγ4 chain with a Vδ6-bearing chain. Twenty-five of the twenty-six hybridomas having this in-frame *V* gene combination exhibited PPD-reactivity; it is not yet clear why the remaining hybridoma (BNT-11.54) was nonreactive. Thus, whereas specificity for both the antigen and the presenting molecule seems to be encoded by the germline *V* gene segments, the diversity seen in the VD and VDJ joints of reactive hybridomas could serve simply to fine tune the response.

We next attempted to identify the *Vδ* gene segments used in the three *Vδ6*⁻ but PPD-reactive hybridomas (BNT-21.2, 41BNT-24 and 41BNT-117). Based on previous hybridization data[1], we performed PCR analysis with a *Vα2* oligonucleotide. We found that 41BNT-117, as well as two of the non-PPD-reactive hybridomas, possessed an in-frame transcript containing a new *Vδ* gene, which we have designated *Vδ8*. Both the nucleotide sequence and predicted corresponding amino-acid sequence of *Vδ8* (Fig. 3*a* and *b*, respectively) are >90% identical to those of members of the *Vα2* gene family. The Vδ8⁺ PPD-reactive hybridoma expressed an in-frame Vγ1JCγ4 transcript, whereas the two non-reactive Vδ8⁺ hybridomas did not.

A summary of *Vγ* and *Vδ* gene segment use in the 28 PPD-reactive and 25 non-reactive hybridomas is given in Table 1. The absolute requirement for the Vγ1JCγ4 chain in the PPD-reactive hybridomas is conspicuous. Note that the *Vγ2* gene, although nearly 90% identical to *Vγ1* at the predicted amino-acid level[7], was never found to encode the γ-chain expressed

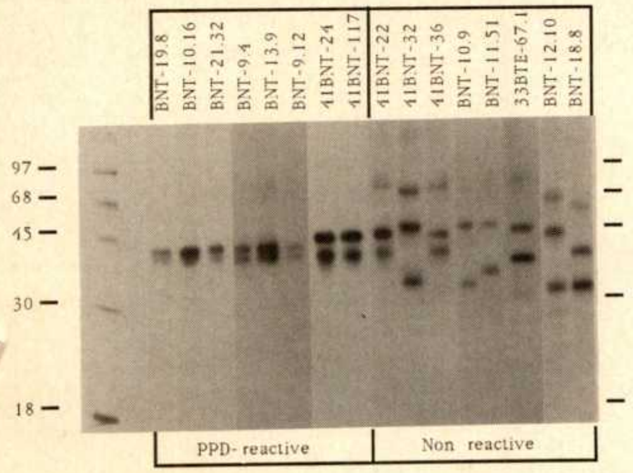

FIG. 1 Examination of surface TCR on γδ hybridomas by SDS-PAGE under reducing conditions. Hybridomas were generated and tested for antigen reactivity as previously described[1,27]. Representatives from both the PPD-reactive and the non-reactive collections are grouped accordingly. Bands corresponding to M_rs of >50,000 (50K) are residual nonreduced heterodimer.

METHODS. Cell-surface radioiodination of T-cell hybridomas was performed using Iodogen (Pierce, Rockville, Illinois) as described previously[28]. Cell lysates were prepared by detergent lysis using 1% digitonin[29]. Immunoprecipitations of the cell lysates were performed as described previously using the anti-CD3 monoclonal antibody HMT3-1 (R.T.K. *et al.*, manuscript in preparation) with protein A-Sepharose (Sigma)[30]. The immunoprecipitates were solubilized by boiling in Laemmli sample buffer[30] containing 0.01 M iodoacetamide, and electrophoresed on a SDS-PAGE gel (10% polyacrylamide). After electrophoresis, the gel was stained, destained, dried and autoradiographed for 18 h. Using the autoradiograph to localize the γδ heterodimer, each γδ band was excised from the dried gel and rehydrated in Laemmli sample buffer containing 5% 2-mercaptoethanol for 1–2 h. The gel slices were loaded onto a second SDS-PAGE gel (10% polyacrylamide) and electrophoresed to resolve the γ- and δ-subunits. The autoradiographs were developed after 3–5-days exposure. The positions of M_r protein standards ($M_r \times 10^{-3}$) are indicated on the left.

a

```
                              10        20        30        40
Vγ1                 QLEQTELSVTRATDESAQISCIVSLPYFSNTAIHWYRQKAKKFEYLIYVS
 31 clones                      --------------------------------------

Vα7.1               EKVIQVWSTASRQEGEELTLDCSYETSQVLYHLFWYKHLLSGEMVFLIR
 Vδ6.3 (23 clones)                   --------------------------------
 Vδ6(pλ12)          QR-T--QP---S-W---V---------EYF-RI---RQ-F--------Y
 5 clones                        W---V---------EY

                    50        60        70        80        90
Vγ1                 TNYNQRPLGGKNKKIEASKDFQTSTSTLKINYLKKEDEATYYCAVW
 31 clones          ----------------------------------------------

Vα7.1               QTSSSTAKERSGRYSVVFQKSLKSISLIISALQPDDSGKYFCALWE
 Vδ6.3 (23 clones)  ----------------------------------------------
 Vδ6(pλ12)          -P-FD-QNQ------------F-----V---S--E---T-----S-
 5 clones                                      --S--E---T-----S-
```

b

Clone	Vδ	N	Dδ1	Dδ2	N	Jδ1	Vγ1	N	Jγ4
9.12	ALWE	L	WH	IGGIR	A	TDKLV	YCAVW		SGTSW
23.4	----	H		-----	-	-----	-----		-----
10.7	----	H		-----	-	-----	-----	T	----
9.4;23.2	----	H		-----	-	-----	-----	I	----
40BNS-8	---	A		-----	-	-----	-----		----
13.6	----	R		-----	-	-----	-----	I	----
23.16	----	-V		----	-	----	-----		-----
3.12;18.13;23.12	----	-V		----	-	-----	-----	I	----
4.1	----	-V		----	-	-----	-----		-----
13.17;15.4;15.15	----	-		---	-	-----	-----	I	----
8.29	----	-		---	-	-----	-----		-----
*18.8	----	-		--	-	-----	(not transcribed)		
10.16	--S	N		-----	-	-----	-----		----
21.26	--S-	-		-----	-	-----	-----	IT	----
*13.13	--S-	-		-----	-	-----	(non-functional)		
18.17	--S-	-		----	-	-----	-----	I	----
19.8	----	-Y		RRDT	T	-----	-----	IA	----
11.6	----	-D		RRDT	T	-----	-----		----
18.5	----	-D		RR	D	-----	-----	IR	-----
*11.54	----	-AG		DT	-	-----	-----	Y	----
14.5	----	H		R	SA	-----	-----	I	----
21.32	--S-	KD	GIY	RR	D	-----	-----	Y	----
23.21	----	-		SEG	Y	----	-----	IS	---

FIG. 2 *a*, Predicted amino-acid sequences encoded by the *Vγ1* and *Vδ6* gene segments (single-letter amino-acid code). Nucleotide sequences are identical to those previously published for *Vγ1* (ref. 6), *Vα7.1* (ref. 10) and *Vδ6(pλ12)* (ref. 12). Vδ6.3 and Vδ6(*p*λ12) have 69% amino-acid identity; the *Vδ6.3* gene segment is more closely related to the *Vδ6.1* (refs 8, 12; 86% amino-acid identity) and *Vδ6.2* (ref. 12; 97% amino-acid identity) family members. *b*, Predicted amino-acid sequences of the V–J junctional regions of the δ- and γ-chain mRNA found in the 28 Vδ6⁺ hybridomas. Hybridomas are from the 'BNT' collection (ref. 27; for example, BNT-9.12) unless otherwise noted. Hybridomas marked with an asterisk do not demonstrate PPD reactivity. No γ-chain junctional sequence is shown for those hybridomas which do not possess a functional *Vγ1JCγ4* transcript (BNT-18.8 and BNT-13.13). This γ-chain gene is not rearranged in the BW fusion line[14]. All clones bearing identical amino-acid sequences in the junctions were identical at the nucleotide level as well.

METHODS. RNA was isolated as previously described[31]. PCR amplification and sequence analysis of the amplified products was performed as previously described[32] using the following oligonucleotide primers (5′ to 3′): 5′*Vα7–Vδ6* external, GGATCTAATGTGGCCGAGA or GGATCTAATGTGGCCCAGA (amino-acid position, −4 to +3); 5′*Vα7* internal, AAGTGATTCAGGTCTGGTCA (amino-acid position, +3 to +9); 5′*Vδ6* internal, AGAAAGTGACTCAGGTCCAG (amino-acid position, +2 to +8); 5′*Cδ* external (antisense), ACAAGCAACATTTGTTCCAT (amino-acid position, +8 to +15 of *Cδ*); 5′*Cδ* internal (antisense), TTTTCATGATGAAACAGAT (amino-acid position, +15 to +21 of *Cδ*); 5′ *Vγ1* external, GGGCTTGGGCAGCTGGAGCA (amino-acid position, −3 to +4); 5′ *Vγ1* internal, AGTATCTAATATATGTCTCA (amino-acid position, +1 to +7); 5′ *Cγ4* external (antisense), GAAGGAAGGAAAATAGTAGG (amino-acid position, +12 to +18 of *Cγ*); 5′ *Cγ4* internal (antisense), GGAGAAAAGTCTGAGTCAGT (amino-acid position, +4 to +10 of *Cγ*).

FIG. 3 Nucleotide sequences (*a*) and corresponding predicted amino-acid sequences (*b*) of *Vα2.1* (isolated from C57BL/Ka (ref. 10) but identical to C57BL/10 (M.P.H., D. DiGiusto and E.P., unpublished observation)), *Vα2.2* (isolated from BALB/c mice; M. Zauderer, personal communication) and *Vδ8* (designated *Vδ7* in ref. 1 just before a different *Vδ7* sequence was described in ref. 14; isolated from C57BL/10 and C57BL/6 strain mice).

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a
                                       10                            20                            30
Vα2.1(TA39) CAGCAGGTGAGACAAAGTCCCCAATCTCTGACAGTCTGGGAAGGAGAGACAGCAATTCTGAACTGCAGTTATGAGGACAGCACTTTTGACTAC
Vα2.2(pFGB) ---------------------------------------------------------------------------------------A-----
Vδ8                                     -----------------------------------A---------------A----TG-----------

                                    40                            50                            60
Vα2.1       TTCCCATGGTACTGGCAGTTCCCTAGGGAAAGCCCTGCACTCCTGATAGCCATACGTCCAGTGTCCAATAAAAAGGAAGATGGACGATTCACAATCTTC
Vα2.2       ------------CA---C------G-----G-----------------T-----A--T--------G--------------------------------
Vδ8         ------------CA----------G-----G-------T------------------T--------G--------------------------------

                           70                            80                            90
Vα2.1       TTCAATAAAAGGGAGAAAAAGCTCTCCTTGCACATCACAGACTCTCAGCCTGGAGACTCAGCTACCTACTTCTGTGCAGCAAAT
Vα2.2       --------------------------------------------------------------C-------------------G-
Vδ8         --------------------------------------------------------------C--T----------------G-

b
                     10        20        30        40        50        60        70        80        90
Vα2.1       QQVRQSPQSLTVWEGETAILNCSYEDSTFDYFPWYWQFPRESPALLIAIRPVSNKKEDGRFTIFFNKREKKLSLHITDSQPGDSATYFCAAN
Vα2.2       -----------------------------N-----QN--G-G-----S-SS--D-------------------------------------S
Vδ8                 -----------------N-A-------Q---G-G--------S--D-------------------------------------S
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METHODS. As in Fig. 2, with the addition of the following *Vα2* oligonucleotides (5′ to 3′): 5′ *Vα2* external, AGCAGCAGGTGAGACAAAGT (amino-acid position, +1 to +7); 5′ *Vα2* internal, CCCCAATCTCTGACAGTCTG (amino-acid position, +8 to +14).

in any of the PPD-reactive cells. But the *Vγ2* gene segment has only been found to rearrange the *JCγ2* gene cluster, which differs considerably from the *JCγ4* gene cluster to which the *Vγ1* gene segment generally rearranges. Thus the use of *Vγ1JCγ4* can be dictated as much by the need for the *JCγ4* gene segments as by the need for the *Vγ1* gene segment. It is clear that not all *Vδ* gene segments are permissive for PPD-reactivity, but Table 1 does include at least four that are: *Vδ6.3*, *Vδ6* (pλ12), *Vδ8* and at least one more unidentified *Vδ*.

The *Vδ6*-expressing hybridomas that we analysed represent all of the *Vδ6*-expressing surface receptor-bearing cells that we have so far generated; they are all derived from newborn thymus and are not a subset selected on the basis of reactivity. The apparent 'predisposition' of these cells to express a specific γ-chain gene (26 out of 28 express *Vγ1JCγ4*, see Table 1) indicates that this is another instance of restricted γ-δ-chain pairing[12,15,16]. In support of this observation, a *Vδ6*-bearing hybridoma generated by McConnell *et al.*[12] expressed a Vγ1Cγ4 protein, and several T-cell lines producing Vδ6 mRNA also expressed the *Vγ1Cγ4* gene product. The tendency for these two chains to be associated with one another could simply be due to physical constraints, or to the ontogenetic timing of *V* gene rearrangements. A third possibility, supported by the fact that the frequently appearing Vδ6–Vγ1JCγ4 combination produces a defined antigen specificity, is that the limited pairing seen is due to a selective pressure to generate this antigen specificity (that is, 'functional constraints').

Finally, the PPD-reactivity of most hybridomas expressing Vγ1JCγ4 is reminiscent of the ability of certain *Vβ* gene segments to confer reactivity to *Mls* gene products or staphylococcal enterotoxins (the proposed 'superantigens') in 80–90% of the $\alpha\beta^+$T-cells in which they are expressed[17-23]. Such $\alpha\beta^+$T-cell responses are unusual in that they seem to involve recognition of relatively nonpolymorphic determinants of MHC gene products. Some $\gamma\delta^+$ T-cell responses involve specific MHC class I molecules (refs 24, 25), or *TL* (ref. 24) or *Qa* (ref. 26) gene products, but the presenting molecule for mycobacterial antigens has so far not been identified. This molecule seems to be relatively nonpolymorphic in some instances[1,3] but not in others[4,5]. It remains to be seen whether the use of only one *Vγ* gene in all of the PPD-reactive hybridomas indicates that PPD represents a superantigen for these cells, or that the cells all recognize one particular antigenic epitope or presenting molecule, but the observations presented here should be useful in elucidating the nature of antigen recognition by the γδTCR. □

TABLE 1 Summary of *Vγ* and *Vδ* gene usage in PPD-reactive and non-reactive $\gamma\delta^+$ T-cell hybridomas

Vγ→ Vδ↓	PPD-reactive		Non-reactive	
	Vγ1Jγ4	Other *Vγ*	*Vγ1Jγ4*	Other *Vγ*
Vδ6.3	21	0	1	1
Vδ6 (*p*λ*12*)	4	0	0	1
Vδ8	1	0	0	2
Vδ1	0	0	0	10
Other *Vδ*	2	0	2	8
Total	28	0	3	22

Vγ gene usage is classified as *Vγ1Jγ4* or non-*Vγ1Jγ4* as determined by sequencing of PCR amplification products (see Fig. 2 legend). The use of *Vδ6.3*, *Vδ6*(*p*λ*12*) or *Vδ8* was determined first by RNA hybridization analysis[1] and then by sequencing of PCR amplification products (see Fig. 2 legend). The use of *Vδ1* was determined by RNA hybridization analysis only[1], therefore not all may be rearranged in frame. Unidentified *Vδ* genes are grouped as 'other *Vδ*'.

Note added in proof. A small portion of a probable *Vδ*, isolated from a thymocyte circular DNA library[33], is closely related to the *Vδ8* gene we describe here.

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1. O'Brien, R. L. *et al. Cell* **57**, 667–674 (1989).
2. Janis, E. M., Kaufman, S. H. E., Schwartz, R. H. & Pardoll, D. M. *Science* **244**, 713–716 (1989).
3. Holoshitz, J., Konig, F., Coligan, J. E., DeBruyn, J. & Strober, S. *Nature* **339**, 226–229 (1989).
4. Modlin, R. L. *et al. Nature* **339**, 544–548 (1989).
5. Haregewoin, A., Soman, G., Hom, R. C. & Finberg, R. W. *Nature* **340**, 309–312 (1989).
6. Marusic-Galesic, S. *et al. J. Immun.* **142**, 28–34 (1989).
7. Iwamoto, A. *et al. J. exp. Med.* **163**, 1203–1212 (1986).
8. Chien, Y. *et al. Nature* **330**, 722–727 (1987).
9. Yague, J. *et al. Nucleic Acids Res.* **16**, 11355–11364 (1988).
10. Arden, B., Klotz, J. L., Siu, G. & Hood, L. E. *Nature* **316**, 783–787 (1985).
11. Elliott, J. F., Rock, E. P., Patten, P. A., Davis, M. M. & Chien, Y. *Nature* **331**, 627–631 (1988).
12. McConnell, T. J. *et al. J. Immun.* **142**, 2924–2931 (1989).
13. Chien, Y., Iwashima, M., Kaplan, K., Elliott, J. & Davis, M. *Nature* **327**, 677–682 (1987).
14. Takagaki, Y., Nakanishi, N., Ishida, I., Kanagawa, O. & Tonegawa, S. *J. Immun.* **142**, 2112–2121 (1989).
15. Korman, A. J., Marusic-Galesic, S., Spencer, D., Kruisbeek, A. M. & Raulet, D. H. *J. exp. Med.* **168**, 1021–1040 (1988).
16. Asarnow, D. M. *et al. Cell* **55**, 837–847 (1988).
17. Kappler, J. W., Staerz, U., White, J. & Marrack, P. C. *Nature* **332**, 35–40 (1988).
18. MacDonald, H. R. *et al. Nature* **332**, 40–45 (1988).
19. Kanagawa, O., Palmer, E. & Bill, J. *Cell. Immun.* **119**, 412–426 (1989).
20. Pullen, A., Marrack, P. & Kappler, J. W. *Nature* **335**, 796–801 (1988).
21. Abe, R., Vacchio, M. S., Fox, B. & Hodes, R. J. *Nature* **335**, 827–830 (1988).
22. White, J. *et al. Cell* **56**, 27–35 (1989).
23. Janeway, C. A., Chalupny, J., Conrad, P. J. & Buxser, S. *J. Immunogenet.* **15**, 161–168 (1988).
24. Bluestone, J. A., Cron, R. Q., Cotterman, M., Houlden, B. A. & Matis, L. E. *J. exp. Med.* **168**, 1899–1916 (1988).
25. Rivas, A., Koide, J., Cleary, M. L. & Engelman, E. G. *J. Immun.* **142**, 1840–1846 (1989).
26. Vidovic, D., Roglic, M., McKune, K., Guerder, S., MacKay, C. & Dembic, Z. *Nature* **340**, 646–649 (1989).
27. Happ, M. P. & Palmer, E. *Eur. J. Immun.* **19**, 1317–1325 (1989).
28. Kubo, R. T. & Pelanne, M. L. *Molec. Immun.* **20**, 67–76 (1983).
29. Oettgen, H. C., Pettey, C. L., Maloy, L. E. & Terhorst, C. *Nature* **320**, 272–275 (1986).
30. Haskins, K. *et al. J. exp. Med.* **157**, 1149–1169 (1983).
31. Kumagai, N., Benedict, S., Mills, G. & Gelfand, E. *J. Immun.* **139**, 1393–1399 (1987).
32. Bill, J. *et al. J. exp. Med.* **169**, 115–133 (1989).
33. Okazaki, K. & Sakano, H. *EMBO J.* **7**, 1669–1674 (1988).

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The colony stimulating factor-1 receptor associates with and activates phosphatidylinositol-3 kinase

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COLONY stimulating factor-1 (CSF-1) is a lineage-specific growth factor required for proliferation and survival of mononuclear phagocytes and their precursors. The CSF-1 receptor belongs to a family of ligand-activated protein-tyrosine kinases. Activation of the platelet-derived growth factor receptor, but not the CSF-1 receptor, leads to an increase in phospholipase C activity and a subsequent elevation in intracellular calcium. Recent studies have shown that a novel phosphoinositol (PtdIns) kinase, termed PtdIns-3 kinase, is stimulated by the platelet-derived growth factor receptor and certain oncogenes in the protein-tyrosine kinase family. PtdIns-3 kinase phosphorylates the D-3 hydroxyl position of the inositol ring of PtdIns, and its products do not participate in the generation of the second messenger inositol 1,4,5-trisphosphate ($Ins(1,4,5)P_3$). Here we report that addition of CSF-1 is followed by activation of PtdIns-3 kinase in a macrophage cell line (P388 D1), which contains CSF-1 receptors, and in BALB/c fibroblasts made to express the human CSF-1 receptor. Furthermore, we show that activation of the CSF-1 receptor results in the accumulation in intact cells of polyphosphoinositides phosphorylated at the D-3 position of the inositol ring. Thus activation of the CSF-1 receptor stimulates PtdIns-3 kinase activity, indicating a novel pathway for CSF-1 receptor-mediated signal transduction.

CSF-1 activity is mediated through a single class of high-affinity receptors[1-3]. The gene encoding the CSF-1 receptor (c-*fms*) is the normal cellular homologue of the v-*fms* oncogene and belongs to a family of structurally related transmembrane growth-factor receptors with ligand-activated protein-tyrosine kinase activity. Other members of this family include the receptor for platelet-derived growth factor (PDGF) and the c-*kit* gene product, the ligand for which is unknown. These receptors are unique among protein-tyrosine kinases in that their kinase domains contain an insert of 70–100 amino acids. Although the PDGF and CSF-1 receptors are structurally similar, there are differences in the cellular responses that follow their activation. For example, PDGF activates PtdIns turnover and increases intracellular calcium[4,5], whereas CSF-1 does not affect PtdIns turnover or cytosolic calcium in murine macrophages[6].

Recent studies have identified a unique PtdIns kinase activity associated with certain activated tyrosine kinases[7,8]. This PtdIns kinase, called PtdIns-3 kinase, physically associates with $pp60^{v\text{-}src}$ and polyoma middle T/$pp60^{c\text{-}src}$ complexes[9], and is also detected in antiphosphotyrosine immunoprecipitates from cells transformed with v-*src*, v-*fms* and v-*abl*, and in quiescent fibroblasts and smooth muscle cells after stimulation with PDGF[7,10,11]. PtdIns-3 kinase phosphorylates PtdIns at the D-3 position of the inositol ring of PtdIns to form a novel product, phosphatidylinositol 3-phosphate (PtdIns(3)P)[12].

We investigated whether PtdIns-3 kinase is activated in response to CSF-1. Human CSF-1 receptor was expressed in murine fibroblasts at $\sim 0.5\text{–}1 \times 10^4$ receptors per cell. Full-length complementary DNA clones for the human CSF-1 receptor gene and feline v-*fms* were introduced into recombinant retrovirus pLJ (ref. 13). BALB/c fibroblast cell lines expressing these constructs were generated by retroviral infection as previously described[14]. Immunoblot analysis was used to screen cells for *fms*-encoded proteins (Fms) and the expression of CSF-1 receptor protein was confirmed by [^{35}S]methionine labelling and immunoprecipitation with monoclonal anti-Fms antibodies. Fibroblasts make CSF-1, but no autocrine effect was observed because murine CSF-1 does not stimulate human CSF-1 receptor, although human CSF-1 stimulates human and murine receptors[15-17]. Therefore, no alterations of cell growth or serum requirements were detected in the c-*fms*-expressing cells.

PtdIns kinase activity was detected in immunoprecipitates of CSF-1-stimulated cells (Fig. 1). Confluent serum-starved BALB/c fibroblasts which express CSF-1 receptor were stimulated with human recombinant CSF-1, lysed and immunoprecipitated with anti-phosphotyrosine antibodies. The greatest production of phosphatidylinositol phosphate (PtdInsP) was observed when c-*fms*-expressing cells were stimulated with 20 ng ml^{-1} of human recombinant CSF-1 (results not

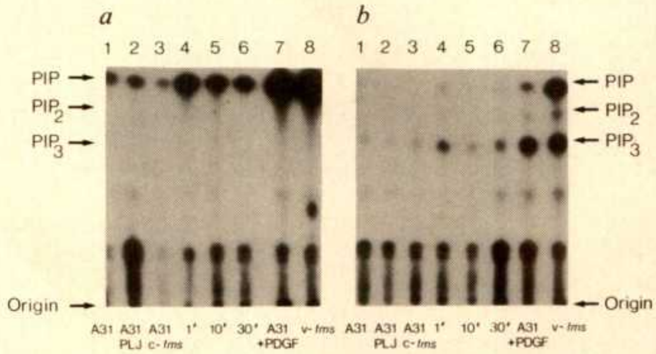

FIG. 1 Time course of CSF-1 dependent activation of PtdIns kinase in anti-phosphotyrosine immunoprecipitates. *a*, TLC analysis of products of lipid kinase assay of anti-phosphotyrosine immunoprecipitates using PtdIns as substrate: A31 parent BALB/c fibroblast cell line (lane 1); A31 cells transfected with the vector alone (lane 2); quiescent A31 fibroblasts expressing human CSF-1 receptor (lane 3); A31 cells expressing human CSF-1 receptor stimulated for 1,10 and 30 min with 20 ng ml^{-1} of human recombinant CSF-1 (lanes 4, 5 and 6); A31 cells expressing human CSF-1 receptor stimulated for 10 min with 10 ng ml^{-1} of recombinant PDGF-BB (lane 7); and v-*fms*-transformed fibroblasts (lane 8). Identification of the product was performed using commercial PtdInsP (PIP) as standard. The migration of the standards (visualized by iodine staining) are indicated by the arrows. Phosphoinositide formation in lanes 1–3 was identified as PtdIns(4)P after deacylation and HPLC analysis. *b*, TLC analysis of the products of lipid kinase assay of anti-phosphotyrosine immunoprecipitates using $PtdIns(4,5)P_2$ as substrate. Lanes are the same as in *a*. PtdIns and PtdIns(4)P contaminants in the commercial $PtdIns(4,5)P_2$ resulted in some formation of PIP and $PtdInsP_2$ (PIP_2). The migration of $PtdInsP_3$ (PIP_3) standard synthesized from polyoma middle T/$pp60^{c\text{-}src}$ immunoprecipitates is indicated by the arrow. METHODS. Confluent cultures of BALB/c fibroblasts were placed in serum-free DME medium supplemented with 1% BSA for 24 h to induce quiescence. Recombinant human CSF-1 (courtesy of S. Clark, Genetics Institute) was added to cells for indicated times (prime symbols denote min). Cells were lysed in a 25 mM HEPES buffer, pH 7.5, containing 137 mM NaCl, 1 mM EDTA, 200 μm sodium vanadate, 10% glycerol, 1% Nonidet P-40, 10 μg ml^{-1} aprotinin and 1 mM phenylmethylsulphonyl fluoride, and immunoprecipitated by incubation for 4 h with anti-phosphotyrosine monoclonal antibody linked to solid support as described by Frackelton *et al.*[24]. The immunoprecipitates were washed twice with each of the following: (1) ice-cold PBS; (2) 0.5 M LiCl and 0.1 M Tris buffer, pH 7.4; and (3) 0.1 M NaCl, 1 mM EDTA in 0.1 M Tris buffer, pH 7.4 (ref. 9). PtdIns kinase activity was assayed in the immunoprecipitates in the presence of 25 mM HEPES buffer, pH 7.4, and 10 mM $MgCl_2$. A mixture of 0.1 mg ml^{-1} PtdIns (*a*) or PIP_2 (*b*) and 0.1 mg ml^{-1} phosphatidylserine, dispersed by zonication in 25 mM HEPES buffer pH 7.4 and 1 mM EDTA, was added to the immunoprecipitates. The reaction was initiated by addition of 20 μCi of [γ-^{32}P]ATP and 100 μM ATP as previously described[12]. The products were separated by TLC in 1-propanol:acetic acid (2*N*) [65:35 (v/v)], visualized by autoradiography and identified by comparison with non-labelled standards.

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shown). This concentration of CSF-1 was used in all subsequent experiments, and is equivalent to 3,000 U ml^{-1} (1U is equivalent to 0.44 fmol); and is comparable to the amount of CSF-1 that elicits a maximal mitogenic response in murine macrophages which express a similar number of CSF-1 receptors[18,19]. Increased immunoprecipitable PtdIns kinase activity was detected within 1 min after addition of CSF-1 (Fig. 1*a*). No CSF-1-dependent PtdIns-3 kinase activity was detected when parent BALB/c fibroblasts or cells infected with the vector alone were used (results not shown). The PldIns-3 kinase activity detected in antiphosphotyrosine immunoprecipitates of PDGF-stimulated cells was fivefold higher than that detected in CSF-1-stimulated cells (lanes 5 and 7, Fig. 1*a*). These activities correspond to the relative abundance of each receptor. Antiphosphotyrosine immunoprecipitates of v-*fms*-transformed cells had elevated PtdIns-3 kinase activity in the absence of exogenous CSF-1 (lane 8, Fig. 1*a*).

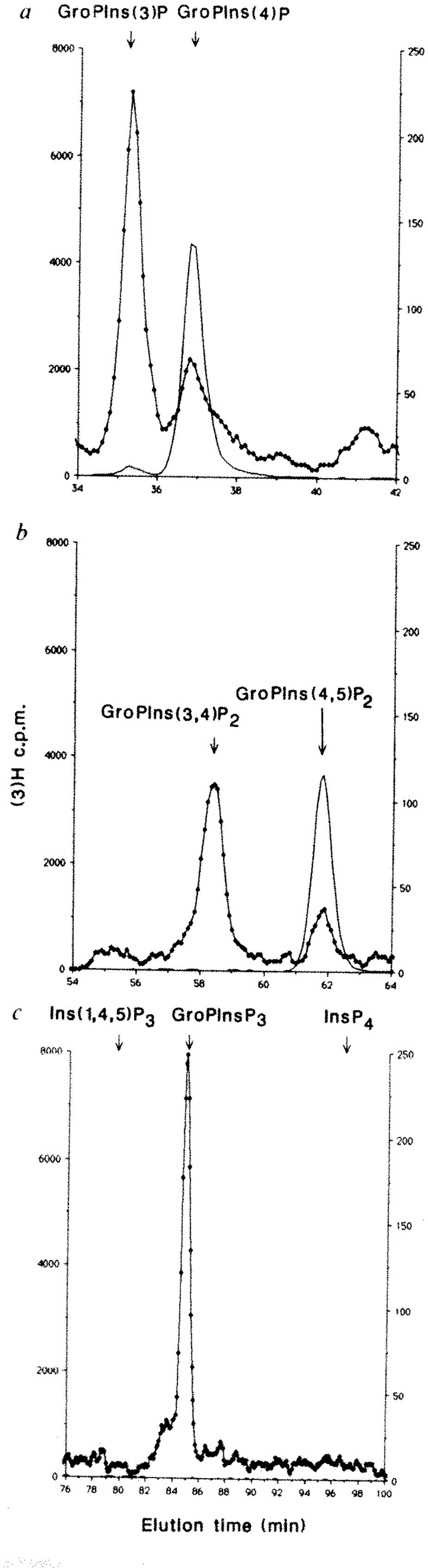

FIG. 2 HPLC identification of polyphosphoinositides generated from antiphosphotyrosine immunoprecipitates of CSF-1-stimulated cells. *a*, HPLC separation of the deacylated product of PtdIns kinase assay of anti-phosphotyrosine immunprecipitates from CSF-1-stimulated cell. The [^{32}P]PtdInsP was produced and deacylated with methylamine reagent as previously described[19]. Solid line represents [^{3}H]GroPIns(4)P standard. ■, Deacylated ^{32}P-labelled PtdInsP generated from fibroblasts which express human CSF-1 receptor after exposure to 20 ng ml^{-1} of human recombinant CSF-1 for 5 min. The migration position of GroPIns(3)P was determined from a parallel run of [^{32}P]GroPIns(3)P standard generated from middle T/pp60$^{c\text{-}src}$ immunoprecipitates. *b*, HPLC separation of the deacylated product generated as above except using PtdIns(4)P as substrate. Solid line represents [^{3}H]GroPIns(4,5)P$_2$ standard. Closed squares represent deacylated ^{32}P-labelled PtdInsP$_2$. The migration position of the GroPIns(3,4)P$_2$ was determined from a parallel run of [^{32}P]GroPIns(3,4)P$_2$ standard generated from middle T/pp60$^{c\text{-}src}$ immunoprecipitates. *c*, HPLC separation of the deacylated product generated as above, except using PtdIns(4,5)P$_2$ as substrate. Closed squares represent deacylated ^{32}P-labelled PtdInsP$_3$. Arrows indicate the migration position from a parallel run of [^{3}H]Ins1,4,5P$_3$ and [^{3}H]Ins1,3,4,5P$_4$ standards (NEN), and the position of [^{32}P]GroPInsP$_3$ generated from middle T/pp60$^{c\text{-}src}$ immunoprecipitates.

METHODS. Deacylated phospholipids were suspended in 400 μl H$_2$O with appropriate standards and separated by anion exchange HPLC using a Partisphere Sax column with a gradient from 0 to 1.0 M (NH$_4$)$_2$HPO$_4$ (pH 3.8) developed over 130 min[11]. Isotope detection was performed using an on-line continuous flow scintillation detector (Radiomatic Instruments) which separates ^{3}H, ^{32}P counts, and a parallel reading of UV channel for detection of non-labelled ADP and ATP standards.

Antiphosphotyrosine-antibody immunoprecipitates from CSF-1-stimulated cells also had lipid kinase activities which used phosphatidylinositol 4-phosphate (PtdIns(4)P) and phosphatidylinositol 4,5-biphosphate (PtdIns (4,5)P$_2$) as substrates. Figure 1b presents the results of an experiment in which PtdIns (4,5)P$_2$ was used as a substrate. The product of this reaction migrated with phosphatidylinositol trisphosphate (PtdInsP$_3$) standard on TLC. A significant increase in PtdIns(4,5)P$_2$ kinase activity was detectable within 1 min (the decrease in activity seen at 10 min in Fig. 1*b*, lane 5, was not reproduced in three other experiments). Elevated PtdIns(4,5)P$_2$ kinase activity was also detected in immunoprecipitates from v-*fms*-transformed cells and in BALB/c control cells stimulated with PDGF. When PtdIns(4)P was used as a substrate, a PtdIns P$_2$ was produced with a similar CSF-1 dependence (data not shown). HPLC identification of the products obtained in these reactions is shown in Fig. 2. The lipids were deacylated and identified by comparison of their HPLC-migration positions with those of standards: [^{32}P]PtdIns(3)P, [^{32}P]PtdInsP$_2$ and [^{32}P]PtdInsP$_3$ standards were synthesized using middle T/pp60$^{c\text{-}src}$ immunoprecipitates[19]; [^{3}H]PtdIns(4)P and [^{3}H]PtdIns(4,5)P$_2$ standards were from a commercial source. No D-3 phosphorylated polyphosphoinositides were detected in control lanes. The deacylation product of the PtdInsP which was produced by antiphosphotyrosine immunoprecipitates from CSF-1-stimulated cells comigrated with glycerophosphoinositol 3-phosphate (GroPIns(3)P); the deacylated PtdInsP$_2$ comigrated with glycerophosphoinositol 3,4-bisphosphate (GroPIns(3,4)P$_2$) standard, and the deacylated PtdInsP$_3$ comigrated with glycerophosphoinositol-trisphosphate (GroPInsP$_3$) standard. Thus, CSF-1 receptor seems to stimulate the same lipid kinase activities as those associated with middle T/pp60$^{c\text{-}src}$ and PDGF receptor[19].

A monoclonal antibody which specifically recognizes the N-terminal domain of both *c-fms* and v-*fms* proteins was used to investigate whether PtdIns-3 kinase directly associates with the CSF-1 receptor. Increased phosphorylation of PtdIns was detected in immunoprecipitates from CSF-1-stimulated cells when anti-Fms antibodies were used. HPLC analysis of the deacylated lipid showed that although some PtdIns(4)P was produced by the immunoprecipitates, the principal lipid was PtdIns(3)P. A similar result was detected using immunoprecipitates from v-*fms*-transformed cells (Fig. 3). These results indicate that PtdIns-3 kinase is physically associated with ligand-activated CSF-1 receptor and with v-Fms oncoprotein.

We also investigated the ability of CSF-1 to stimulate PtdIns-3 kinase in a cell line which expresses endogenous CSF-1 receptor. P388 D1 is a murine macrophage cell line which expresses 3.5×10^3 CSF-1 receptors per cell[2]. After addition of recombinant human CSF-1, an increase in PtdIns-3 kinase activity was detected in anti-phosphotyrosine immunoprecipitates (data not shown). Thus, activation observed with the transfected receptor is not merely a consequence of expression of the receptor in fibroblasts.

The polyphosphoinositides produced by PtdIns-3 kinase in immunoprecipitates from CSF-1-stimulated cells were also elevated in response to CSF-1 in intact cells. P388 D1 macrophage cells were starved of serum for 14 h and labelled with $^{32}PO_4^{3-}$ for 3 h. Analysis of deacylated phospholipids from starved murine macrophage cells showed small but detectable amounts of ^{32}P-labelled lipids which comigrated with deacylated PtdIns(3)P, PtdIns(3,4)P_2 and PtdInsP_3 standards. These lipids each represented, respectively, 3.2%, 0.7% and 0.2% of the ^{32}P label incorporated into PtdIns(4)P in the same HPLC analysis. This observation differs from results obtained from serum-starved fibroblasts and smooth muscle cells where no PtdIns(3,4)P_2 or PtdInsP_3 was detected[19], and could be due to the difficulty in synchronizing these macrophages into a G_0 state. Addition of human recombinant CSF-1 for 5 min resulted in a 70% increase in PtdIns(3,4)P_2, and a 300% increase in PtdInsP_3. The levels of these lipids declined but remained above control values at 30 min (Fig. 4). No CSF-1-mediated changes in the content of PtdIns(3)P were seen. Similar results were observed in smooth muscle cells[11] after addition of PDGF, suggesting that intracellular PtdIns(3)P levels do not change during mitogenic response.

The physiological significance of PtdIns-3 kinase remains to be determined. None of the products of this kinase are in the pathway for generating the Ca^{2+}-regulating second messenger, Ins(1,4,5)P_3, suggesting that these lipids provide signals independent of the conventional PtdIns turnover response. Evidence for the importance of PtdIns-3 kinase for growth and transformation is provided by studies of mutants for the polyoma middle T gene and mutants for the PDGF receptor gene. In most middle T gene mutants which are defective in cell transformation, PtdIns-3 kinase fails to associate with the middle T/pp60$^{c\text{-}src}$ complex[9,20]. Included in this group of transformation-defective genes are mutants in which the middle T protein associates with and activates the protein-tyrosine kinase activity of pp60$^{c\text{-}src}$, but fails to associate with or activate PtdIns-3 kinase. A similar phenotype has been observed for a mutant PDGF receptor in which 83 of the 100 amino acids in the protein-

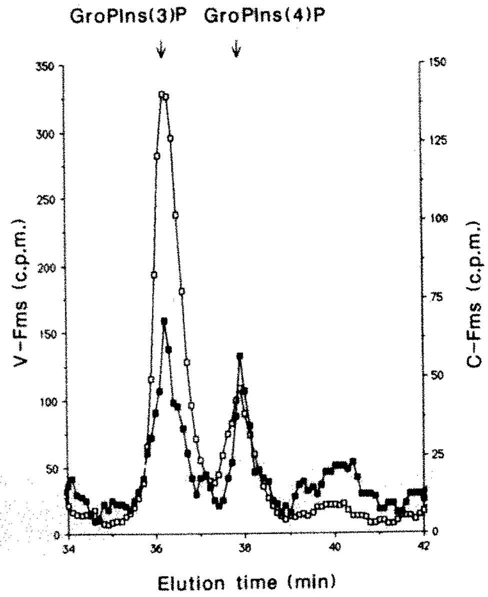

FIG. 3 HPLC analysis of the deacylated PtdInsP product produced by the anti-Fms immunoprecipitates of c-Fms expressing fibroblasts which were stimulated with CSF-1- and v-Fms-transformed cells. ■, Deacylated ^{32}P-labelled PtdInsP generated from immunoprecipitates of *c-fms*-expressing cells stimulated with CSF-1. □, Deacylated ^{32}P-labelled PtdInsP generated from immunoprecipitates of v-*fms*-transformed cells (no CSF-1 added). CSF-1-stimulated fibroblasts which express the CSF-1 receptor and v-*fms*-transformed cells were lysed, equalized for the amount of protein, and reacted with rat monoclonal anti-Fms antibody for 6 h. An anti-rat polyclonal antibody linked to a solid support (Cooper Diagnostics) was used as a second antibody. PtdInsP generated by immunoprecipitable PtdIns kinase was analysed as indicated in Fig. 2.

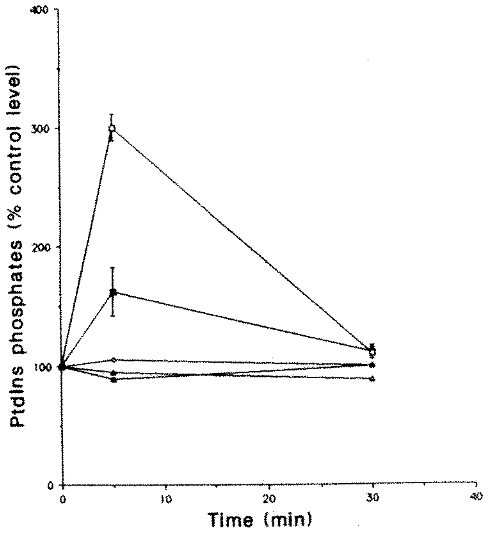

FIG. 4 Time course of the levels of polyphosphoinositides in intact murine macrophages after stimulation with CSF-1: *In vivo* levels of PtdInsP_3 (□); PtdIns(3,4)P_2 (■); PtdIns(3)P (△); PtdIns(4)P (◇) and PtdIns(4,5)P_2 (▲) are expressed as percentages of the control level. Each point represents three independent experiments. The error bars represent s.d. Murine macrophages were grown to confluency in RPMI, 10% FCS and deprived of serum for 14 h. Cells were washed with phosphate-free MEM supplemented with 1-glutamine and 1% BSA, and labelled for 3 h with 0.1 mCi $^{32}PO_4^{3-}$ ml^{-1}. The medium was removed, cells were rinsed with the same medium without $^{32}PO_4^{3-}$, and stimulated with 10 ng ml^{-1} human recombinant CSF-1 at time =0. At the end of the periods indicated, the medium was removed and 1 M HCl was added to the plate. Cells were gathered with the aid of a Teflon cell scraper and the phospholipids were extracted into methanol:chloroform (1:2, v/v)[11]. The phospholipids were deacylated as indicated in the legend to Fig. 2 and identified on HPLC by comparison with deacylated standards. Between 0.8 and 2×10^6 ^{32}P c.p.m. were used per HPLC run. In a typical experiment (1×10^6 c.p.m. injected into HPLC), the total counts incorporated at time zero were 177,206 for PtdIns(4)P, 219,936 for PtdIns(4,5)P_2, 5,818 for PtdIns(3)P, 1,252 for PtdIns(3,4)P_2, and 366 for PtdInsP_3.

tyrosine kinase domain insert are deleted[21,22]. This receptor retained PDGF-stimulated protein-tyrosine kinase activity, stimulated PtdIns turnover and calcium elevation, but did not associate with PtdIns-3 kinase and failed to mediate PDGF-dependent DNA synthesis. These results indicate that activation of PtdIns-3 kinase could be essential for the growth and transformation response of these genes. But transformation-defective mutants of middle T protein (ref. 23, and B.D. and T.R., unpublished observations) and of p60$^{v\text{-}src}$ (ref. 20) which retain wild type levels of PtdIns-3 kinase have been found, suggesting tha this activation alone is not sufficient for transformation. W have shown that the CSF-1 receptor has similar properties t the PDGF receptor with respect to activation of the PtdIns- kinase pathway, indicating that production of D-3 phosphory lated phosphoinositides could be critical for the growth-stimu lating activities of these two receptors.

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1. Stanley, E. R. & Jubinsky, P. T. *Clin. Haemat.* **13**, 329–348 (1984).
2. Guilbert, L. J. & Stanley, E. R. *J. biol. Chem.* **261**, 4024–4032 (1986).
3. Guilbert, L. J. Tynan, P. W. & Stanley, E. R. *J. cell. Biochem.* **31**, 203–216 (1986).
4. Habenicht, A. J. R. *et al.*, *J. Biol. Chem.* **256**, 12329–12235 (1981).
5. Berridge, M. Heslop J. Irvine R. F. & Brown, K. D. *Biochem. J.* **222**, 195–201 (1984).
6. Whetton, A. D. Monk P. N. Consalvey, S. D. & Downes, C. P. *EMBO J.* **5**, 3281–3286 (1986).
7. Whitman, M. Kaplan, D. Roberts, T. & Cantley, L. *Biochem. J.* **247**, 165–174 (1987).
8. Kaplan D. R. *et al Cell* **50**, 1021–1029 (1987).
9. Whitman, M. Kaplan, D. R. Schaffhausen, B. Cantley, L. and Roberts, T. *Nature* **315**, 239–242 (1985).
10. Varticovski, L. & Cantley, L. *Blood* **72**, 232a (1988).
11. Auger, K. R. Serunian, L. A. Soltoff, S. P. Libby, P. & Cantley, L. *Cell* **57**, 167–175 (1989).
12. Whitman, M. Downs, C. P. Keeler, M. Keller, T. & Cantley, L. *Nature* **332**, 644–646 (1988).
13. Morrison, D. K. Browning, P. White, M. & Roberts, T. *Molec. cell. Biol.* **8**, 176–185 (1988).
14. Piwnika-Worms, H. Saunders, K. B. Roberts, T. M. Smith, A. E. & Cheng, S. H. *Cell* **49**, 75–82 (1987
15. Das, S. K. Stanley, R. E. Guilbert, L. J. & Forman, L. W. *J. cell. Physiol.* **104**, 359–364 (1980).
16. Russel, M. F. *et al. Nature* **325**, 549–552 (1987).
17. Rousel, M. F. Downing, J. R. Rettenmier, C. W. & Sherr, C. J. *Cell* **55**, 979–986 (1988).
18. Stanley, E. R. & Guilbert, L. J. *J. immunol. Meth.* **42**, 253–284 (1981).
19. Morgan, C. Pollard, J. W. & Stanley, R. *J. cell. Physiol.* **130**, 420–427 (1987).
20. Fukui, Y. & Hanafusa, H. *Molec. cell. Biol.* **9**, 1651–1658 (1989).
21. Coughlin, S. R. Escobedo, J. A. & Williams, L. T. *Science* **243**, 1191–1193 (1989).
22. Williams, L. T. *Science* **243**, 1564–1570 (1989).
23. Morgan, W. C. Kaplan, D. R. Pallas, D. C. & Roberts, T. M. *J. Virol.* **62**, 3407–3411 (1988).
24. Frackleton, A. R. Jr, Ross, A. H. & Eisen, N. H. *Molec cell. Biol.* **3**, 1343–1352 (1983).

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Differential expression of genes encoding α, β and γ retinoic acid receptors and CRABP in the developing limbs of the mouse

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RETINOIC acid has profound effects on vertebrate limb morphogenesis (refs 1–6, reviewed in refs 7–9), including in the mouse, where it can act as a teratogen generating phocomelia and bone defects[10–12]. A retinoic acid gradient[13–15], possibly amplified by a graded distribution of cellular retinoic acid-binding protein (CRABP)[16], could provide positional information across the antero-posterior axis of the chick limb bud. The discovery of nuclear retinoic acid receptors (RARs)[17–22] acting as retinoic acid-inducible enhancer factors (reviewed in refs 23, 24) provided a basis for understanding how retinoic acid signals could be transduced at the level of gene expression[25]. We have now used *in situ* hybridization to study the distribution of messenger RNA transcripts of the three murine receptors (mRARs) and CRABP during mouse limb development. Both mRARα and mRARγ transcripts, but not those for mRARβ, are present and uniformly distributed in the limb bud at day 10 post-coitum, whereas CRABP transcripts have a graded proximo-distal distribution, indicating that differential expression of CRABP, but not of mRARα or mRARγ, could participate in the establishment of the morphogenetic field. At later stages, mRARγ transcripts become specific to the cartilage cell lineage and to the differentiating skin and mRARβ transcripts are mostly restricted to the interdigital mesenchyme. CRABP transcripts, however, are excluded from regions expressing mRARγ and mRARβ. These results indicate that all three RARs and CRABP have specific functions during morphogenesis and differentiation of the mouse limb.

We synthesized ^{35}S-labelled antisense RNA probes from mRARα, mRARβ and mRARγ (ref. 21) and murine CRABP (ref. 26) complementary DNA clones, and hybridized them to serial sets of contiguous histological sections. Three stages of the mouse limb development were studied, corresponding to nondifferentiated limb buds (day 10 post-coitum (p.c.)), the appearance of precartilaginous blastemas (day 12.5 p.c.) and the beginning of ossification and digit separation (day 14.5 p.c.). Forelimb bud sections at day 10 p.c. showed that mRARγ (Fig. 1*a*, *b*) and mRARα (data not shown) transcripts were homogeneously distributed throughout the limb bud. The signal for mRARγ transcripts stopped at the proximal limit of the buds, whereas that for mRARα transcripts extended into adjacent tissues. mRARβ transcripts were detected only in the most proximal region of the limb buds and in the mesenchyme of the flank (data not shown). Whereas CRABP transcripts were abundant and uniformly distributed in posterior sections of the forelimb (Fig. 1*a*, R), there was a gradual proximal-to-distal increase in transcripts in more anterior sections (Fig. 1*a*, L; *b*), so that no labelling was seen in the proximal core of the bud, whereas it was maximal at the distal tip. No CRABP transcripts were detected in the ectodermal layer (Ec), except in the apical ectodermal ridge (AER), where only the most dorsal cells were repeatedly labelled, irrespective of their antero-posterior location (Fig. 1*a*–*c*). By contrast, both the AER and ectoderm were devoid of mRARγ transcripts (Fig. 1*c*). The immunohistochemistry study of Maden *et al.*[16] has similarly revealed a higher concentration of CRABP in the distal progress zone of the chick limb bud. Moreover, the existence of an antero-posterior gradient of CRABP in this zone, with opposite polarity to that of retinoic acid[13] has been reported[16]. We did not find an equivalent gradient of CRABP transcripts. Further studies are required to establish whether such a gradient of CRABP exists. The results described here, however, indicate that there could be a proximo-distal gradient of CRABP, which is maximal distally. This could create a gradient of free retinoic acid across the limb bud, which may be interpreted by the uniformly expressed mRARα or mRARγ, or both, at the time when positional information is to be specified.

By day 12.5 p.c., a remarkable segregation of the domains of expression of mRARγ and CRABP was apparent. Murine RARγ transcripts were proximally restricted to central precartilaginous blastemas (PB) of both forelimb (Fig. 1*d*) and hindlimb (Fig. 1*e*), and more homogeneously distributed in the distal undifferentiated mesenchyme of both limbs. By marked contrast, CRABP transcripts appeared to be excluded from the central PB and were most abundant in cells underlying the ectoderm

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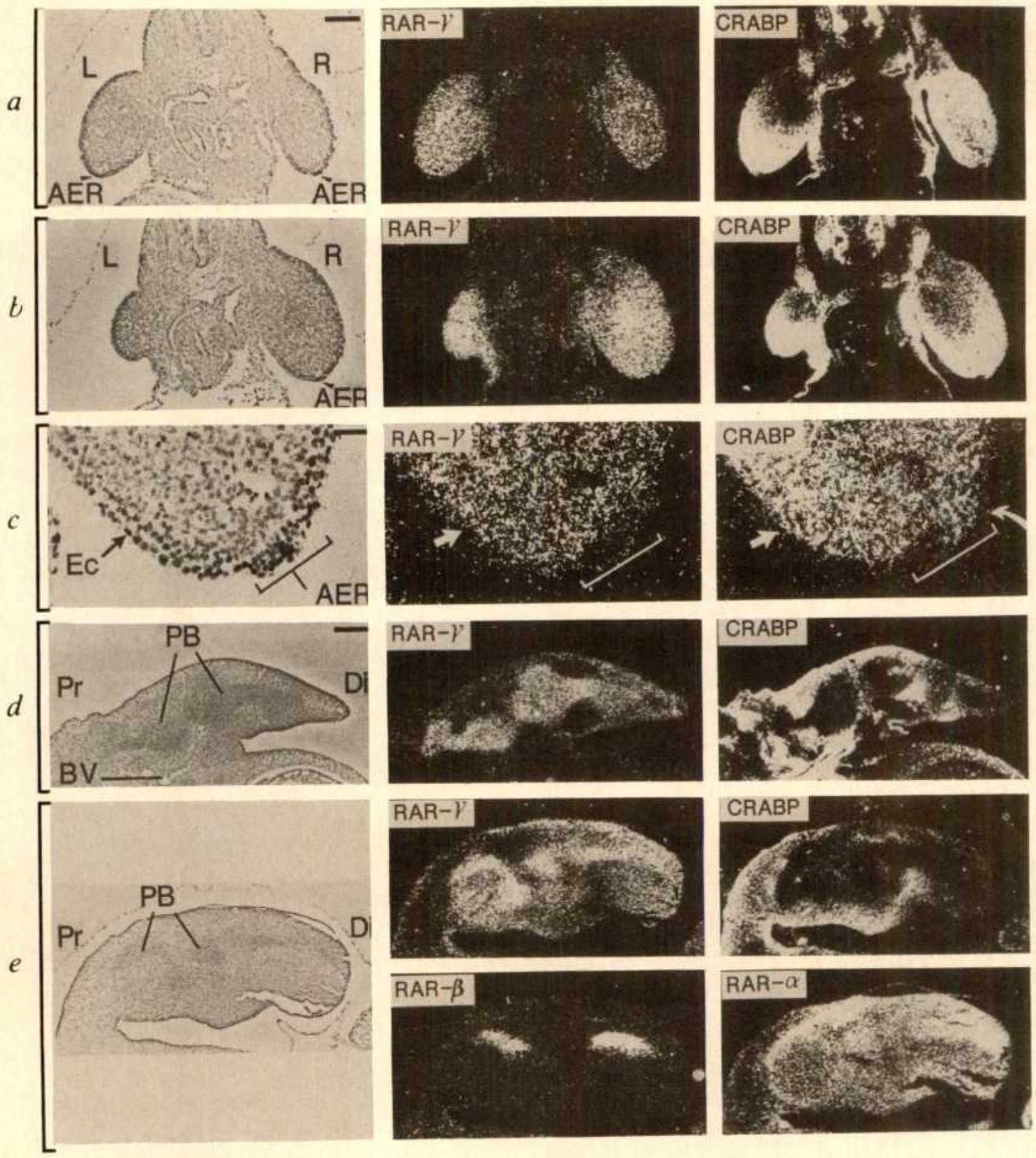

IG. 1 Detection of mRARs and CRABP transcripts in the developing murine limbs by gestational days 10 and 12.5 p.c. Bright-field views of selected section planes are shown in left panels for histological identification. The right and middle panels are dark-field views showing autoradiography signal (white grains) on contiguous sections hybridized with mRARs and CRABP probes, as indicated. Sections hybridized to CRABP probes were exposed half as long as all others. *a, b*, Two serial transverse sections across the forelimb buds of a 10-day-p.c. embryo. The section shown in *b* is more anterior than in that shown in *a*. Because the section plane is slightly biased, the left limb (L) is cut more anteriorly. Bar, 160 μm. *c*, Higher magnification of the distal region of the forelimb bud (including the AER as indicated) of a 10-day-p.c. embryo. Note that absence of labelling in the ectoderm (Ec) with both probes (the short white arrows point to the same Ec position in the dark-field views as the black arrow in the bright-field view), and the selective labelling of the dorsal part of the AER with the CRABP probe (curved arrow). Bar, 50 μm. *d*, Longitudinal section of a 12.5-day-p.c. forelimb. Bar, 200 μm. *e*, Sagittal section of a 12.5-day-p.c. hindlimb extremity; the footplate points to the right. L, left limb bud; R, right limb bud; AER, apical ectodermal ridge; Ec, ectoderm; Pr, proximal; Di, distal; PB, precartilaginous blastemas; BV, blood vessels.

METHODS. Murine RARα, mRARβ, mRARγ (ref. 21) and CRABP (ref. 26) full-length cDNAs subcloned into plasmid pSG5 (ref. 34) in an antisense orientation were used as templates in a T7 polymerase *in vitro* transcription reaction. α-^{35}S-labelled CTP (Amersham) was added to the reaction to yield antisense RNA probes of specific activity ~5.10^{8} c.p.m. μg^{-1}. Probe average length was reduced to ~100 nucleotides by limited alkaline hydrolysis. Collection, paraformaldehyde fixation, paraffin embedding and sectioning of mouse embryos were carried out as described in ref. 35. The *in situ* hybridization protocol was previously described[35], except that the prehybridization step was omitted. Contiguous sections (5-μm thick) were hybridized respectively to each of the four probes, in a sequentially repeated manner so that 50-μm regularly spaced section planes were analysed. Emulsion autoradiography exposures were for 14 days for mRARs probes, and for 7 days for the CRABP probe. Specificity controls included hybridization of parallel sections to α-^{35}S-labelled dATP tailed oligonucleotide probes corresponding to regions of least homology across the mRARs. These probes resulted in the same signal distribution as that obtained with riboprobes, but longer exposure times were required.

(Fig. 1*d, e*) and surrounding the limb axial blood vessels (Fig. 1*d*, BV), with very little CRABP expression in the distal mesenchyme where mRARγ transcripts were homogeneously distributed. At the same stage, mRARα transcripts were widely distributed (Fig. 1*e*), whereas mRARβ transcripts (Fig. 1*e*) were only expressed in a discrete proximal region of the hindlimb mesenchyme, distinct but adjacent to the zeugopod PB, and in several regions of loosely packed cells in the forefoot and hindfoot plates, similar to that shown in Fig. 1*e*.

By day 14.5 p.c., mRARγ transcripts were found exclusively in the epidermal and dermal layers of the fetal skin (Fig. 2*a, d*) and in the cartilage cells of every bone model (Fig. 2*a, b*), with maximal expression in their growing extremities (for example, that of humerus in Fig. 2*a*) and no significant expression in ossification centres. Nevertheless, mRARγ labelling remained more diffuse in the extremities of the fingers (Fig. 2*c*) where bones, muscles and skin had not yet differentiated (see legend to Fig. 2). By contrast, the mRARα gene was expressed in all tissues including muscle, skin and perichondrium, but at lower levels in cartilage (Fig. 2*a–d*). Murine RARβ transcripts were highly restricted to mesenchymal cells located in the interdigital regions (Fig. 2*b, c*). CRABP transcripts were selectively distributed at the periphery of cartilaginous bone models and in the dermis, but not in the epidermal layer, nor in muscles (Fig. 2*a–d*). It is interesting that CRABP transcripts were not detected in the precise regions of the digits where the genes encoding mRARβ and mRARγ were maximally expressed, although they were clearly seen in the surrounding mesenchyme (Fig. 2*b, c*).

The fact that the genes encoding the three RARs are differentially expressed at the time of cytodifferentiation in the murine

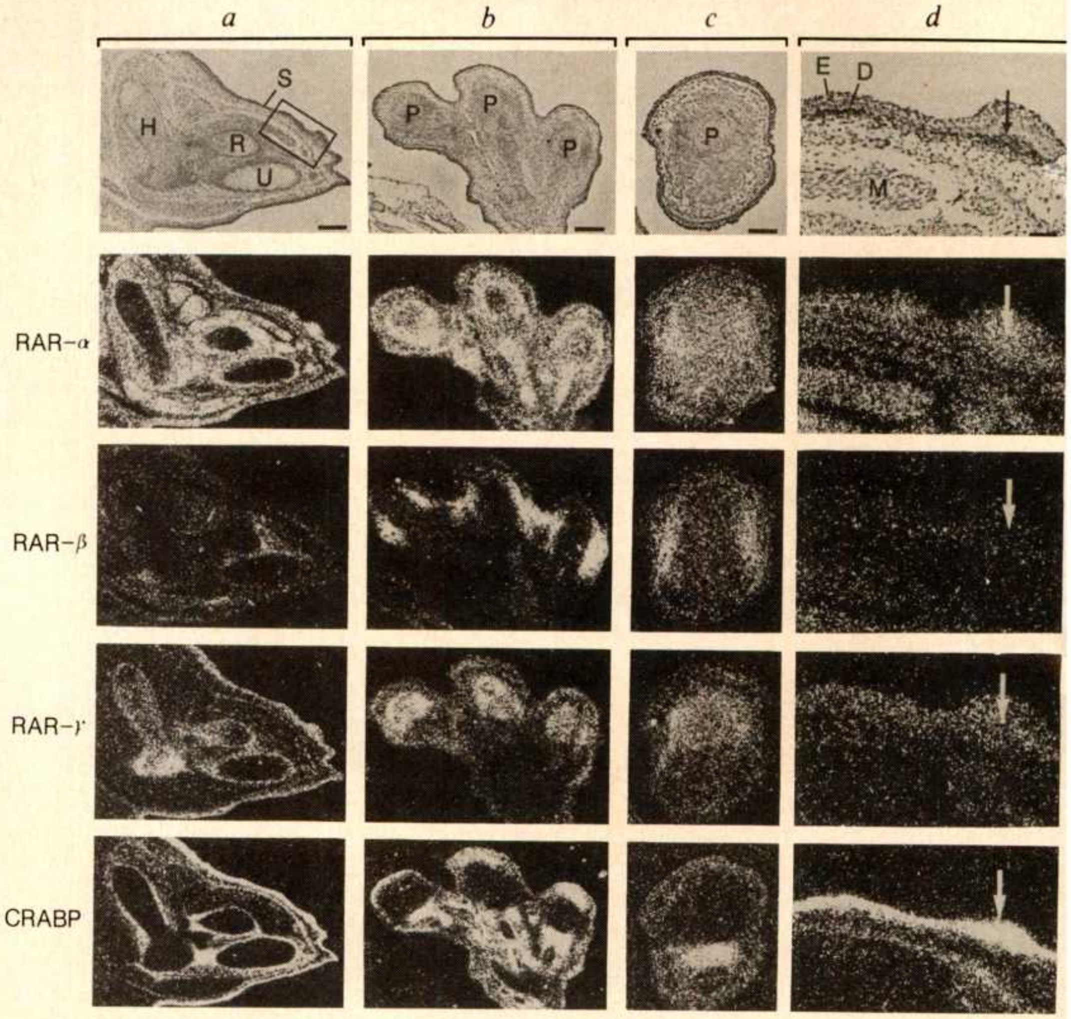

FIG. 2 Distribution of mRARs and CRABP transcripts in the limbs by gestational day 14.5 p.c. Top panels are bright-field views and lower panels show dark-field views of contiguous sections hybridized to mRARα, mRARβ, mRARγ and CRABP probes as indicated. *a*, Sagittal section of the elbow region, crossing the fetal humerus, radius and ulna. Note that at this stage of development the distal region of the humerus (H) and the proximal region of the radius (R) which are seen in this section contain both cartilaginous areas (lower part in the humerus, left part in the radius) and an ossification centre (upper part in the humerus, right part in the radius), whereas the section crosses the ulna essentially in an ossification centre. Bar, 200 μm. *b*, Section across the extremity of the hindlimb, crossing three digits. Bar, 160 μm. *c*, Transverse section of a forelimb finger extremity, just distal to the interdigital cleft. The distal regions of the fingers are still poorly differentiated; this is particularly clear in the case of the skin where the epidermis and dermis are not yet differentiated (compare with upper panel of *d*) and for the central condensation where chondrogenesis is not yet complete. The mRARβ signal is restricted to the mesenchyme facing the adjacent digits, whereas the mRARγ signal is more evenly distributed, although enhanced in the central condensation. Bar, 100 μm. *d*, Higher magnification of the region of the forelimb boxed in *a*. The arrows point to the basal lamina separating the dermis (D) and the epidermis (E); mRARα labelling appears ubiquitous (including muscles (M), whereas no significant mRARβ labelling is seen, mRARγ appears to be expressed both in dermis and epidermis, and CRABP is expressed in the dermis to such a great extent that the autoradiography grain is visible on the bright-field view (top panel), but not in the epidermis or in muscles. Bar, 75 μm. H, humerus; R, radius; U, ulna; S, skin; P, phalangeal bone anlagen; E, epidermis; D, dermis; M, muscle.

limb, strongly supports the idea that each RAR performs specific functions[21,22]. The specific localization of mRARγ transcripts in the cartilaginous cell lineage and in the differentiating skin, which extends throughout the embryo[27], indicates that mRARγ could mediate the effects of retinoic acid on chondrogenesis[11,28,29] and on skin differentiation[30]. The expression of the gene encoding mRARβ could be associated with programmed cell death[31], because it is highly restricted to the mesenchyme of the interdigital regions at the time of digit separation, and to their possible precursor areas in the differentiating footplates. CRABP transcripts are generally excluded from the areas expressing the mRARβ and mRARγ genes (although they are present in the surrounding regions) and to a large extent from those areas expressing the gene encoding mRARα. Thus, it seems possible that CRABP is not directly involved in the transduction of the retinoic acid signal by RARs. Whether it has a role in the control of free retinoic acid levels as previously suggested by its gradient distribution in the chick limb bud[16] is unknown. It is noteworthy that CRABP transcripts are preferentially localized in the skin dermal layer, whereas the genes encoding mRARα and mRARγ are expressed in both dermis and epidermis, because skin differentiation could be programmed by progressive migration of keratinocytes away from the dermal vascular source of retinoids[32,33]. □

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1. Maden, M. *Nature* **295,** 672-675 (1982).
2. Thoms, S. D. & Stocum, D. L. *Devl Biol.* **103,** 319-328 (1984).
3. Scadding, S. R. & Maden, M. *J. embryol. exp. Morphol.* **91,** 35-53 (1986).
4. Tickle, C., Alberts, B. M., Wolpert, L. & Lee, J. *Nature* **296,** 564-565 (1982).
5. Summerbell, D. *J. Embryol. exp. Morphol.* **78,** 269-289 (1983).
6. Tickle, C., Lee, J. & Eichele, G. *Devl Biol.* **109,** 82-95 (1985).
7. Maden, M. *Trends Genet.* **1,** 103-107 (1985).
8. Brockes, J. P. *Neuron* **2,** 1285-1294 (1989).
9. Eichele, G. *Trends Genet.* **5,** 246-251 (1989).
10. Kochhar, D. M. *Teratology* **7,** 289-299 (1973).
11. Satre, M. A. & Kochhar, D. M. *Devl Biol.* **133,** 529-536 (1989).
12. Sulik, K. K., Johnston, M. C. & Dehart, D. B. *Teratology* **35,** 32A (1987).
13. Thaller, C. & Eichele, G. *Nature* **327,** 625-628 (1987).
14. Slack, J. M. W. *Nature* **327,** 553-554 (1987).
15. Thaller, C. & Eichele, G. *Development* **103,** 473-483 (1988).
16. Maden, M., Ong, D. E., Summberbell, D. & Chytil, F. *Nature* **335,** 733-735 (1988).
17. Petkovich, M., Brand, N. J., Krust, A. & Chambon, P. *Nature* **330,** 444-450 (1987).
18. Giguere, V., Ong, E. S., Segui, P. & Evans, R. M. *Nature* **330,** 624-629 (1987).
19. Brand, N. *et al.* *Nature* **332,** 850-853 (1988).
20. Benbrook, D., Lernhardt, E. & Pfahl, M. *Nature* **333,** 669-672 (1988).
21. Zelent, A., Krust, A., Petkovich, M., Kastner, P. & Chambon, P. *Nature* **339,** 714-717 (1989).
22. Krust, A., Kastner, P., Petkovich, M., Zelent, A. & Chambon, P. *Proc. natn. Acad. Sci. U.S.A.* **86,** 5310-5314 (1989).
23. Green, S. & Chambon, P. *Trends genet.* **4,** 309-314 (1988).
24. Evans, R. M. *Science* **240,** 889-895 (1988).
25. Robertson, M. *Nature* **330,** 420-421 (1987).
26. Stoner, C. M. & Gudas, L. J. *Cancer Res.* **49,** 1497-1504 (1989).
27. Ruberte, E. *et al.* *Development* (in the press).
28. Gallandre, F. & Kistler, A. *Wilhelm Roux Arch. dev. Biol.* **189,** 25-33 (1980).
29. Kochhar, D. M., Penner, J. D. & Tellone, C. *Teratog. Carcinog. Mutagen.* **4,** 377-387 (1984).
30. Shapiro, S. S. in *Retinoids and Cell Differentiation* (ed. H. I. Sherman) 30-55 (1985).

31. Menkes, B. & Ilies, A. *Revue rom. embryol. Cytol.* **2**, 161-172 (1965).
32. Kapan, R., Traska, G. & Fuchs, E. *J. cell. biol.* **105**, 427-440 (1987).
33. Kapan, R. & Fuchs, E. *J. cell Biol.* **109**, 295-307 (1989).
34. Green, S., Issemann, I. & Scheer, E. *Nucleic Acids Res.* **16**, 369-372 (1988).
35. Dollé, P. & Duboule, D. *Embo J.* **8**, 1507-1515 (1989).

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Mutations in the *p53* gene occur in diverse human tumour types

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THE *p53* gene has been a constant source of fascination since its discovery nearly a decade ago[1,2]. Originally considered to be an oncogene, several convergent lines of research have indicated that the wild-type gene product actually functions as a tumour suppressor gene[3-9]. For example, expression of the neoplastic phenotype is inhibited, rather than promoted, when rat cells are transfected with the murine wild-type *p53* gene together with mutant *p53* genes and/or other oncogenes[3,4]. Moreover, in human tumours, the short arm of chromosome 17 is often deleted (reviewed in ref. 10). In colorectal cancers, the smallest common region of deletion is centred at 17p13.1 (ref. 9); this region harbours the *p53* gene, and in two tumours examined in detail, the remaining (non-deleted) *p53* alleles were found to contain mutations[9]. This result was provocative because allelic deletion coupled with mutation of the remaining allele is a theoretical hallmark of tumour-suppressor genes[11]. In the present report, we have attempted to determine the generality of this observation, that is, whether tumours with allelic deletions of chromosome 17p contain mutant *p53* genes in the allele that is retained. Our results suggest that (1) most tumours with such allelic deletions contain *p53* point mutations resulting in amino-acid substitutions, (2) such mutations are not confined to tumours with allelic deletion, but also occur in at least some tumours that have retained both parental 17p alleles, and (3) *p53* gene mutations are clustered in four 'hot-spots' which exactly coincide with the four most highly conserved regions of the gene. These results suggest that *p53* mutations play a role in the development of many common human malignancies.

To search for mutations, two approaches were used, both based on the polymerase chain reaction[12] (PCR) (Fig. 1). For tumour cell lines and for xenografts passaged in athymic nude mice, complementary DNA was generated from messenger RNA using oligo(dT) as a primer. A 1,300 base-pair (bp) fragment including the entire *p53* coding region was generated from the cDNA using PCR, and this fragment was cloned and sequenced in its entirety. For primary tumours, sufficient RNA was often not available for the first approach, and PCR was used to generate a 2.9-kilobase (kb) fragment from tumour DNA. This was the longest fragment that we could reproducibly amplify from the *p53* locus, and included all of the exons found to contain mutations through the first approach.

Using these approaches, we analysed *p53* sequences of tumours derived from the breast, lung, brain, colon or mesenchyme. Tumours of these types have been previously shown to exhibit frequent deletions of chromosome 17p when studied by restriction-fragment length polymorphism (RFLP) methods[10]. To test for allelic deletions, tumour DNA samples were digested with *Hin*fI and, following Southern transfer, hybridized sequentially to two probes (p144D6 (ref. 13) and pYNZ22.1 (ref. 14)) detecting variable-number tandem-repeat ('VNTR' or 'minisatellite') sequences. DNA samples from normal tissues exhibited two alleles with at least one of these probes in 29 of 31 different individuals tested. Because of this high degree of polymorphism, allelic loss could be assessed with greater than 95% certainty in cell lines and xenografts, even when corresponding normal tissue was not available for comparison.

Nineteen tumours with allelic deletions of chromosome 17p were selected for sequence analysis. Thirteen of the tumours were found to contain a single missense mutation, two tumours each contained two missense mutations, one tumour contained a frame-shift mutation at codon 293, and no mutation was detected in three tumours (Table 1). The PCR reaction is known to be associated with a relatively high rate of base misincorporation[12], and we confirmed this observation by noting several sequence variants (13 out of 34,000 bp sequenced) in individual clones that were not reproducibly present in other PCR reactions from the same tumour sample. All of the mutations listed in Table 1 were confirmed by performing a second PCR reaction and re-sequencing the products *en masse* as described in the legend to Fig. 2.

Two observations indicated that the nucleotide substitutions described in Table 1 represented somatic mutations. First, none of these presumptive mutations have been observed in the sequences of human *p53* genes derived from normal cells, SV40-transformed fibroblasts, or lymphoblastoid cell lines (ref. 15, and references therein). Second, in six cases (tumours 2, 3, 9, 12, 13, 16), normal tissue from the patients whose tumours are described in Table 1 were available for study. To test for the presence of the presumptive mutations in the germline of these patients, a strategy was devised that used both PCR and cloning. Although direct sequencing of PCR products has been shown to be possible by several methods, we found that none of the published methods could be reproducibly applied to all parts of the *p53* coding region. To circumvent this difficulty, we cloned the PCR products into a phagemid vector and used the DNA pooled from 10^3 to 10^4 independent phage clones as a template for DNA sequencing (see legend to Fig. 2). This procedure resulted in sequence data quality as high as that produced using individual plasmid DNA clones as templates, and was used to demonstrate that in each of the six cases noted above, the mutations in the tumour DNA were not present in the germline of the patient (examples in Fig. 2).

The data described above indicated that most tumours with one *17p* allele contained a mutation of the *p53* gene in the remaining allele. To begin to assess the status of tumours that had not lost a 17p allele, we examined cDNA clones from three such tumours. In each case, two cDNA clones derived from PCR products, generated as described in the legend to Fig. 1, were sequenced. In one case (tumour 11), both clones contained a single point mutation at codon 134 (Table 1). In the second case (tumour 16), one clone contained a point mutation at codon 281 and one clone was wild-type. In the third case (tumour 17), both clones were wild-type. To assess the relative expression levels of the mutant alleles, the sequencing strategy described

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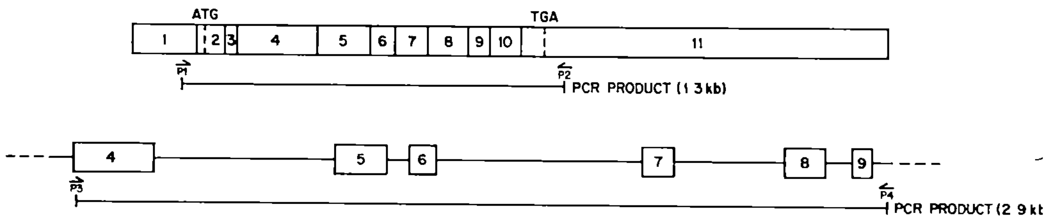

FIG 1 Strategies for amplification of *p53* gene sequences Messenger RNA was used to generate a cDNA template for a polymerase chain reaction (PCR) using primers P1 and P2 (top) The PCR product was 1 3 kb long and included the entire coding region Alternatively, total genomic DNA was used in a PCR reaction using primers P3 and P4 The PCR product was 2 9 kb long and included exons 4–9 (bottom) The numbered boxes indicate exons and the vertical dotted lines indicate the start (ATG) and stop (TGA) codons respectively

METHODS RNA was purified using guanidium isothiocyanate and mRNA selected by binding to Messenger Affinity Paper (Amersham) Complementary DNA was synthesized from 500–750 ng of mRNA using oligo(dT) as a primer The oligo(dT) primer was removed by isopropanol precipitation, 10 μg of transfer RNA and sodium perchlorate (to a final aqueous concentration of 0 5 M) were added to the reaction, and this was followed by addition of 1/2 volume of isopropanol[27] The cDNA was pelleted by centrifugation for 15 min at room temperature and used in a 50-μl PCR reaction consisting of 35 cycles of 93 °C (1 min), 58 °C (1 min), and 70 °C (2 min) Genomic DN (2 μg) was used in a 200-μl PCR reaction consisting of 30 cycles at 95 ° (1 min), 58 °C (1 min), and 70 °C (4 min) PCR reactions contained magnesiu chloride at a final concentration of 2 mM The primers used were P 5′-GGAATTCCACGACGGTGACACG-3′, P2, 5′-GGAATTCAAAATGGCAGGGG AGGG-3′, P3, 5′-GTAGGAATTCGTCCCAAGCAATGGATGAT-3′, P4, 5′-CATCGA ATTCTGGAAACTTTCCACTTGAT-3′ All primers had extraneous nucleotide comprising *Eco*RI sites at their 5′ ends to facilitate cloning The PCR product were digested with *Eco*RI, fractionated by electrophoresis, and followin purification from agarose, ligated to *Eco*RI digested Bluescript vector (Strategene) Individual clones were sequenced with primers derived fro the p53 coding and intron sequences[15] using T7 polymerase

in the legend to Fig 2 was used using cDNA from tumour mRNA as a template In tumour 11, only the mutant allele was expressed (data not shown), in tumour 16, the mutant and wild-type alleles were expressed at approximately equal levels (Fig 2, panel 4)

The fact that three (tumours 5, 8 and 10) of the 19 tumours with single chromosome 17p alleles did not contain *p53* gene mutations has potentially important practical and conceptual implications At least three explanations could account for this result First, it is possible that mutations existed in these three tumours but were outside the region sequenced Such mutations could affect the transcription of *p53* mRNA, its stability or translational capacity Experiments to test this possibility are in progress, but it is notable that although tumour 5 expressed *p53* mRNA, no p53 protein could be detected on western blots (S E Kern and J M N , unpublished data) Alternatively, it is possible that a second tumour suppressor gene on chromosome 17p exists and was the target of allelic deletion in some tumours Finally, the possibility that some allelic deletions represent 'nonselected losses', coincidently occurring with other independent mutations elsewhere in the genome, cannot be excluded

Neurofibrosarcomas are tumours that predominantly occur in patients with neurofibromatosis type I This syndrome can be inherited in an autosomal dominant fashion, and the gene responsible for it (*NF*) has been mapped to chromosome 17q near the centromere[16] Interestingly, chromosome 17 sequences are lost from neurofibrosarcomas from these patients, but the region of deletion sometimes includes the short arm and does not include the region of 17q harbouring the *NF* gene (T G and B Seizinger, unpublished data) The result shown in Table 1 (tumour 12) suggests that a target of allelic deletion, in at least one neurofibrosarcoma, was *p53*

Altogether, 20 point mutations (19 missense, 1 frameshift) were identified in the present study These are mapped in Fig 4, together with the two human *p53* gene missense mutations previously described[9] Several features are notable Although the sample size is limited, the mutations tended to be clustered in four hotspots which accounted for 86% of the 21 missense mutations (five mutations in region A, codons 132–143, five mutations in region B, codons 174–179, three mutations in region C, codons 236–248, five mutations in region D, codons 272–281) There have been two missense mutations identified in murine tumour cells, both in the carcinogen-induced fibrosarcoma cell line Meth A one allele contained a mutation in region A, and the other contained one mutation in region C and one mutation in region D[17,18] Interestingly, the four hotspots for *in vivo* mutation coincided exactly with the four most highly conserved

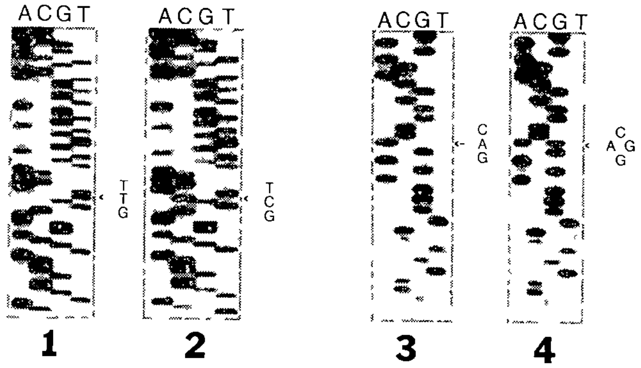

FIG 2 Examples of sequencing reactions demonstrating *p53* gene mutations The templates used for the sequencing reactions shown in panels 1–4 consisted of pools of greater than 10^3 clones generated from PCR products Tumour 13 genomic DNA contained a mutation at codon 239 (antisense GCT, panel 2), instead of the wild-type sequence (GTT) found in the genomic DNA from normal lymphocytes from the same patient (panel 1) Panel 4 shows a sequencing reaction of pooled cDNA clones from tumour 16 showing that both wild-type codon 281 (GAC) and mutant codon 281 (GGC) were both expressed Only the wild-type sequence (GAC) was found in pooled genomic DNA clones from normal lymphocytes of this patient (panel 3)

METHODS PCR reactions were carried out as described in the legend to Fig 1, and the reaction products digested with *Eco*RI The entire reaction was ligated to 0 25 μg of λ ZAP phage vector arms (Stratagene) and packaged using 1/4 of a GIGA-Pack extract (Stratagene) *Escherichia coli* BB4 cells were then infected, and 10^3–10^4 phage clones plated on a 7-cm Petri dish The λ ZAP vector contains the sequences for a phagemid into which the PCR inserts were cloned, and single-stranded DNA phage can be rescued from the λ phage clones using a helper phage[28] An overnight culture of XL-1 Blue cells (Stratagene) was grown in 0 4% maltose and resuspended in 1 5 volumes of 10 mM magnesium sulphate Phages were eluted from the 7-cm dish in 5 ml phage-dilution buffer (100 mM sodium chloride, 10 mM magnesium sulphate, 20 mM Tris, pH 7 5, 0 02% gelatin) for 2 h at room temperature with gentle agitation Fifty microlitres of eluate was used to infect 200 μl of XL-1 Blue cells (Stratagene) in the presence of 1 μl helper phage R408 (10^{11} P F U per ml) After 15 min at 37°, 5 ml of 2 × YT broth was added and the culture shaken for 3 h at 37 °C, then heated to 70 °C for 20 min Cell debris was pelleted at 3,000*g* for 5 min, and 10 μl supernatant, containing single-stranded DNA phage, was used to infect 200 μl of XL-1 Blue cells, prepared as described above After 15 min at 37 °C, 100 μl of the mixture (containing over 10^4 clones determined by titration on XL-1 Blue cells) was inoculated into 50 ml L-broth and shaken overnight at 37 °C Double-stranded DNA was isolated by alkaline lysis and sequenced as described in the legend to Fig 1 The primer used for sequencing in panels 1 and 2 was 5′-GAGGCAAGCAGAGGCTGG-3′ The primer used for sequencing in panels 3 and 4 was 5′-TGGTAATCTACTGGGACG-3′

TABLE 1 *p53* gene mutations in human tumours

Tumour	Tumour name	Tumour type*	Tumour cells tested†	Number of 17p alleles‡	Codon	Mutation	
						Nucleotide	Amino acid
1	D263	Brain	B, X	1	175	CGC→CAC	Arg→His
2	D274	Brain	X	1	273	CGT→TGT	Arg→Cys
3	D303	Brain	B, X	1	216	GTG→ATG	Val→Met
4	D317	Brain	B, X	1	272	GTG→ATG	Val→Met
5	D247	Brain	C	1		None detected	
6	MDA 468	Breast	C	1	273	CGT→CAT	Arg→His
7	T47D	Breast	C	1	194	CTT→TTT	Leu→Phe
8	BT123	Breast	B	1		None detected	
9	1012	Lung	B	1	293	Deleted a G	Frameshift
10	5855	Lung	B	1		None detected	
11	H231	Lung	C	2	134	TTT→TTA	Phe→Leu
12	88-3/14	NFS	B, C	1	179	CAT→TAT	His→Tyr
13	C×4A	Colon	B, X	1	239	AAC→AGC	Asn→Ser
14	C×5A	Colon	X	1	248	CGG→TGG	Arg→Trp
15	C×6A	Colon	X	1	132	AAG→AAC	Lys→Asn
					133	ATG→TTG	Met→Leu
16	C×7A	Colon	B, X	2	281	GAC→GGC	Asp→Gly
17	C×19A	Colon	X	2		None detected	
18	C×20A	Colon	B, X	1	175	CGC→CAC	Arg→His
19	C×22A	Colon	X	1	175	CGC→CAC	Arg→His
20	C×26A	Colon	X	1	141	TGC→TAC	Cys→Tyr
21	SW480	Colon	C	1	273	CGT→CAT	Arg→His
					309	CCC→TCC	Pro→Ser
22	SW837	Colon	C	1	248	CGG→TGG	Arg→Trp

* The brain tumours were glioblastoma multiforme, the colon and breast tumours were adenocarcinomas, the NFS tumour was a neurofibrosarcoma developing in a patient with type-I neurofibromatosis, H231 was a small cell carcinoma of the lung, and the other two lung tumours were non-small-cell carcinomas

† B, Tumour biopsy, C, cell line passaged *in vitro*, X, xenograft derived from biopsy, passaged in athymic nude mice Whenever two sources of tumour cells are listed, both contained the indicated mutation

‡ The number of alleles was determined by RFLP analysis, as described in the text

regions of the *p53* gene, previously identified by Soussi *et al*[19] Of the 41 amino acids contained within regions A–D, 93% are identical in the wild-type *p53* genes of amphibian, avian and mammalian species, compared to a conservation of only 51–57% over the entire *p53* coding sequence The clustering of mutations and evolutionary conservation of regions A–D suggest that they play a particularly important role in mediating the normal function of the *p53* gene product

Previous cytogenetic and RFLP studies have shown that allelic deletions of chromosome 17p occur in at least 60% of tumours of the colon, breast, lung, ovaries, cervix, adrenal cortex, bone and bladder, and in at least 30% of brain tumours[10,20-23] These tumour types account for most of the neoplasms occurring in humans Our data suggest that the great majority of tumours with 17p allelic deletions contain mutant *p53* genes, and similar mutations occur in at least some tumours without 17p allelic deletions

These data lead to the following hypothesis Mutations in the *p53* gene occur during the process of tumorigenesis, and through a dominant negative effect[3-5,9,24], cause tumour progression The dominant negative effect may be mediated by binding of the mutant p53 product to the wild-type product, creating an inactive oligomeric complex[18,25] Because wild-type products remain in the cell, however, a further loss of growth control can be exerted when the wild-type allele is deleted, leaving the cell with only a mutant allele The first example of an intermediate step in this scheme is provided by tumour 16, which had not lost a chromosome 17p allele, but had developed a *p53* gene mutation and expressed both the wild-type and mutant alleles (Table 1 and Fig 2) We imagine that tumour 16, had it not been surgically removed, would have eventually lost the wild-type *p53* gene through allelic deletion Indeed, it has been shown that the loss of a chromosome 17p allele is significantly associated with tumour progression in the human host[26] Although this model requires much further study before it can be verified, it is now supported by several lines of research, including the demonstration of mutant *p53* genes in most tumours with 17p allelic losses ☐

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1 Lane D P & Crawford L. V *Nature* **278,** 261–263 (1979)
2 Linzer, D I H & Levine A J *Cell* **17,** 43–52 (1979)
3 Finlay C A Hinds P W & Levine A J *Cell* **57,** 1083–1093 (1989)
4 Eliyahu D *et al Proc natn. Acad. Sci USA* (in the press)
5 Sturzbecher H-W Addison C & Jenkins J R *Molec. cell Biol* **8,** 3740–3747 (1988)
6 Munroe D G Rovinski B Bernstein A & Benchimol S *Oncogene* **2,** 621–624 (1988)
7 Wolf D & Rotter V *Proc. natn. Acad. Sci USA.* **82,** 790–794 (1985)
8 Masuda H Miller, C Koeffler H P Battifora H. & Cline M J *Proc. natn Acad. Sci USA* **84,** 7716–7719 (1987)
9 Baker S J *et al Science* **244,** 217–221 (1989)
10 Cavenee W K Hastie N D & Stanbridge E J eds *Current Communications in Molecular Biology Recessive Oncogenes and Tumor Suppression* (Cold Spring Harbor Press New York 1989)

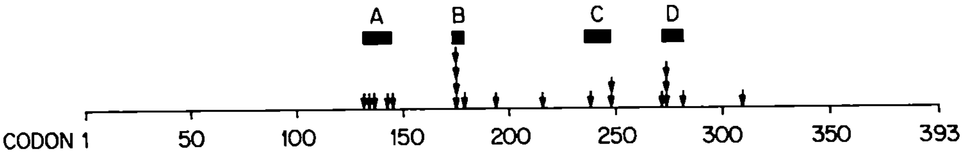

FIG 3 Summary of *p53* point mutations in human cancer Each of the missense mutations listed in Table 1 is indicated with an arrow In addition, the two point mutations described previously[9] in human cancers (at codons 143 and 175) are also included The four regions containing most (86%) of the mutations are indicated by the black bars marked A–D

11 Knudson A *Cancer Res* **45,** 1437–1443 (1985)
12 Saiki R K *et al Science* **239,** 487–491 (1988)
13 Kondoleon S *et al Nucleic Acids Res* **15,** 10605 (1987)
14 Nakamura Y *et al Nucleic Acids Res* **16,** 5707 (1988)
15 Buchman, V L *et al Gene* **70,** 245–252 (1988)
16 Collins F S Ponder B A J Seizinger B R & Epstein C J *Am J hum Genet* **44,** 1–5 (1989)
17 Finlay C A *et al Molec cell Biol* **8,** 531–539 (1988)
18 Eliyahu D *et al Oncogene* **3,** 313–321 (1988)
19 Soussi T *et al Oncogene* **1,** 71–78 (1987)
20 Lee J H *et al Proc Am Ass Cancer Res* **30,** 442 (1989)
21 Atkin N B & Baker M C *Cancer Genet Cytogenet* **37,** 229–233 (1989)
22 Yano T *et al J natn Cancer Inst* **181,** 518–523 (1989)
23 Tsai Y C *et al Cancer Res* (in the press)
24 Herskowitz I *Nature* **329,** 219–222 (1987)
25 Kraiss S Quaiser A Oren M & Montenach M *J Virol* **62,** 4737–4744 (1988)
26 Kern S E *et al J Am Med Ass* **261,** 3099–3103 (1989)
27 Haymerle H *et al Nucleic Acids Res* **14,** 8615–8624 (1986)
28 Short J M Fernandez J M Sorge J A & Huse W D *Nucleic Acids Res* **16,** 7583–7600 (1988)

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Putative transcription activator with alternative isoforms encoded by human *ZFX* gene

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THE *ZFY* gene in the sex-determining region of the human Y chromosome encodes a protein with 13 zinc fingers, and may determine whether an embryo develops as a male or female[1]. *ZFX*, a related gene on the human X chromosome, may also function in sex determination; it encodes a protein with a very similar zinc-finger domain and escapes X inactivation[1,2]. *ZFY* and *ZFX* diverged from a common ancestral gene before the radiation of placental mammals, and retain a similar genomic organization[2]. Analysis of complementary DNAs from the mouse Y-chromosomal homologues of *ZFY* indicates that these genes encode probable transcription activators[3,4] Here, we report that *ZFX* encodes a protein composed of a highly acidic amino-terminal domain, a basic putative nuclear-localization signal, and a carboxy-terminal zinc-finger domain. This combination of features, also found in the *ZFY* gene product, is typical of transcription activators. Alternative splicing generates *ZFX* transcripts encoding isoforms of 575 and 804 amino acids. These ZFX protein isoforms differ in the length of their acidic domains and may be functionally distinct.

ZFX is transcribed in all human cells analysed[2] We cloned *ZFX* cDNAs from a male lymphoblastoid cell line Analysis by restriction mapping and hybridization with genomic probes revealed three distinct types of cDNAs Whereas two types were represented by a single clone each (cDNAs 1 and 3), a third type was represented by three clones (cDNAs 2, 4 and 5)

We determined the nucleotide sequence of one cDNA of each type (Fig 1) Comparison of cDNAs 1, 2, and 3 showed that alternative splicing and polyadenylation had produced structurally distinct 5′ untranslated, coding, and 3′ untranslated regions (Fig 2*a*) Differential splicing involved an invariant donor site (nucleotide −378) and alternative acceptor sites (at nucleotides +647, −28 and −377, in cDNAs 1, 2 and 3, respectively) Each cDNA contains a single long open reading frame (ORF), and in each case the first ATG occurs in a sequence context favourable for initiation of translation[5] Complementary DNAs 2 and 3, with identical ORFs, encode a protein of 804 amino acids (ZFX^{804}), whereas cDNA 1 encodes an isoform of 575 amino acids (ZFX^{575}) (Alternatively, with cDNA 1, translation initiation at a second ATG, whose context is highly favourable, would result in production of an isoform of 573 amino acids) *In vitro* transcription and translation of cDNAs 3 and 1 yielded the predicted full-length and truncated proteins, respectively (data not shown) Both ZFX protein isoforms contain three domains—an N-terminal acidic portion (25% aspartic and glutamic acid), a small basic domain, and a C-terminal run of 13 zinc fingers, each with two cysteines and two histidines (Cys-Cys/His-His zinc fingers) The isoforms differ in that the acidic domain of ZFX^{575} is half that of ZFX^{804}

FIG 1 Composite nucleotide sequence of *ZFX* cDNAs 1, 2 and 3 (Fig 2*a*) and predicted amino-acid sequences Numbering of nucleotides and amino acids is with reference to the first in-frame ATG codon in cDNAs 2 and 3 Known splice sites are indicated by upward arrows Alternative starts of poly(A) tails are indicated by downward arrows Complementary DNA 1 begins at nucleotide −527, uses a splice donor site at −378 and an acceptor site at +647, a tail of 20 adenosines follows nucleotide 2,585 Complementary DNA 2 starts at nucleotide −426, uses the same donor, but a different acceptor at −28, a tail of 46 adenosine residues follows nucleotide 5,450 Complementary DNA 3 starts at nucleotide −426, uses the same donor, but yet another different acceptor at −377, a tail of 26 adenosines follows nucleotide 3,298 Complementary DNAs 2 and 3 contain the same ORF with the first in-frame ATG codon at nucleotide 1, the predicted protein of 804 amino acids is composed of an acidic N-terminal domain, a small basic domain (residues 391–406, boxed), and 13 zinc fingers (cysteines of the Cys-X-X-Cys repeats are circled) Complementary DNA 1 has a shorter ORF with the first in-frame ATG codon at nucleotide +688, encoding a protein of 575 residues The 3′ UTR of cDNA 2 contains an *Alu* sequence[16] (nucleotides +3,959 to +4,250, boxed) An AATAAA polyadenylation signal[17] occurs 20 nucleotides 5′ of the poly(A) tail of cDNA 2 Similar sequences are present 5′ of the poly(A) tails in cDNAs 1 and 3

METHODS Complementary DNA libraries were prepared[3] using poly(A)$^+$ RNA prepared[2] from human male lymphoblastoid cell line WHT1659 Five million recombinant phages from unamplified libraries were screened using plasmids pDP1007, pDP1041 and pDP1006 (*ZFY* genomic fragments which cross-hybridize to *ZFX*)[1,2] as probes Complementary DNA inserts of seven phages were subcloned into Bluescript vectors (Stratagene) The full DNA sequences of three inserts and partial sequences of the other four inserts were determined[2] As judged by comparison with genomic sequences, five cDNAs originated from *ZFX* and two from *ZFY*

Comparison of *ZFX* cDNAs with the genomic locus by restriction mapping and oligonucleotide hybridization gave an overview of the intron-exon organization (Fig 2*a*) The C-terminal zinc-finger domain and 3′ untranslated region (UTR) are encoded by a single exon, whereas the N-terminal acidic domain is encoded by a minimum of four exons for ZFX^{575} (cDNA 1) and a minimum of six exons for ZFX^{804} (cDNAs 2 and 3) The cDNAs span 67 kilobases (kb) in the genome

Of the three types of cDNAs, type 2, encoding ZFX^{804}, seems to be most representative of the 6 3- and 8-kb transcripts observed on northern blots[2] First, the coding exon defined by oligonucleotides ‘150’ and ‘637’ is present in cDNAs 2 and 3 but not in cDNA 1 (Fig 2*a*) Northern analysis using the corresponding genomic DNA fragment revealed that this exon is present in the 6 3- and 8-kb transcripts (not shown) Second, northern analysis indicated that the polyadenylation site used in cDNA 2 corresponds, at least roughly, to the 3′ end of the main *ZFX* transcripts (data not shown) Clone 2, which is 5 6 kb long, could represent a 6 3- or 8-kb transcript that is incomplete at the 5′ end

Transcripts corresponding to cDNA 1, encoding ZFX^{575}, have not been detected by northern analysis Using polymerase chain reaction (PCR) amplification, however, we confirmed the differential splicing predicted from cDNA analysis and crucial to the generation of ZFX protein isoforms (Fig 2*b*) We designed splice-specific 5′ primers spanning splices of the invariant donor site at nucleotide −378 to alternative acceptor sites at nucleotides +647, −28 and −377, as in cDNAs 1, 2 and 3, respectively The

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                                                                 -527 CGGGGAGCTGGGCCGCTTTTTGTCAGCTCCGAGCTCGGCCCCTCCTC
-480 CCTCCCTCCGCCCGCCCCACCAGCCGGAGCCCGGCCCAGTGCTCCAGAGAAAGGCCGGCCTGCAGCACCCGCCACCGTCGCGGCCGCCCGCAACGTCCGTCCGGTTCTTGCTATATTGCC
-360 CCAGGCTGGTCTCGAACTCCTGGGCTCAAGCCGTTCTCCCGCCTCCCACTGCCCGCCTGTCACTGCCGTCTGTTCCCTGAGCTGTCGTTTACGCTGGGAAAGACATAGAAGAACTATTAA
-240 GAAGATAGAATTGTTTTGCTGCGCAGTACAGCAACAGTGGATGTTCAAGATTAAGATTAGAGTCAAGTTGTGTGATTAAGACAGACAGTCTTCCTCTGTCATCCAGGCTGGAATGCAGTG
-120 CAGTGGTGCAAATTTGGCTCACTGCAGCCTCTGCCTTCTGTGTTCAAGTGATTCTTCTTCTCCCTCAGCCTCTCGAGTAGCTGGGATTACAGGAGCTGTGACTGATGAGAATTAAAGGCC
   1 M  D  E  D  G  L  E  L  Q  Q  E  P  N  S  F  F  D  A  T  G  A  D  G  T  H  M  D  G  D  Q  I  V  V  E  V  Q  E  T  V  F
   1 ATGGATGAAGATGGGCTTGAATTACAACAAGAGCCAAACTCATTTTTTGATGCAACAGGAGCTGATGGTACACACATGGATGGTGATCAAATTGTTGTGGAAGTACAAGAAACTGTTTTT
  41 V  S  D  V  V  D  S  D  I  T  V  H  N  F  V  P  D  D  P  D  S  V  V  I  Q  D  V  I  E  D  V  V  I  E  D  V  Q  C  P  D
 121 GTTTCAGATGTTGTGGATTCAGACATAACTGTGCATAACTTTGTTCCTGATGACCCAGATTCAGTTGTAATCCAAGATGTTATTGAGGACGTTGTTATAGAAGATGTTCAGTGCCCAGAT
  81 I  M  E  E  A  D  V  S  E  T  V  I  I  P  E  Q  V  L  D  S  D  V  T  E  E  V  S  L  A  H  C  T  V  P  D  D  V  L  A  S
 241 ATCATGGAAGAAGCAGATGTGTCTGAAACGGTCATCATTCCTGAGCAAGTGCTGGACTCAGATGTAACTGAAGAAGTTTCTTTAGCACATTGCACAGTCCCAGATGATGTTTTAGCTTCT
 121 D  I  T  S  A  S  M  S  M  P  E  H  V  L  T  G  D  S  I  H  V  S  D  V  G  H  V  G  H  V  G  H  V  E  H  V  V  H  D  S
 361 GACATTACTTCAGCCTCAATGTCTATGCCAGAACACGTCTTGACGGGTGATTCTATACATGTGTCTGACGTTGGACATGTTGGACATGTTGGACATGTTGAACATGTGGTTCATGATAGT
 161 V  V  E  A  E  I  V  T  D  P  L  T  T  D  V  V  S  E  E  V  L  V  A  D  C  A  S  E  A  V  I  D  A  N  G  I  P  V  D  Q
 481 GTAGTGGAAGCAGAAATTGTCACTGATCCTCTGACTACCGACGTAGTTTCAGAAGAAGTATTGGTAGCAGACTGTGCCTCTGAAGCAGTCATAGATGCCAATGGGATCCCTGTGGACCAG
 201 Q  D  D  D  K  G  N  C  E  D  Y  L  M  I  S  L  D  D  A  G  K  I  E  H  D  G  S  S  G  M  T  M  D  T  E  S  E  I  D  P
 601 CAGGATGATGACAAAGGCAACTGTGAGGACTACCTTATGATTTCCTTGGATGATGCTGGAAAAATAGAACACGATGGTTCTTCTGGAATGACCATGGACACAGAGTCGGAAATTGATCCT
 241 C  K  V  D  G  T  C  P  E  V  I  K  V  Y  I  F  K  A  D  P  G  E  D  D  L  G  G  T  V  D  I  V  E  S  E  P  E  N  D  H
 721 TGTAAAGTGGATGGCACTTGCCCTGAGGTCATCAAGGTGTACATTTTTAAAGCTGACCCTGGAGAAGATGACTTAGGTGGAACTGTAGACATTGTGGAGAGTGAGCCTGAGAATGATCAT
 281 G  V  E  L  L  D  Q  N  S  S  I  R  V  P  R  E  K  M  V  Y  M  T  V  N  D  S  Q  P  E  D  E  D  L  N  V  A  E  I  A  D
 841 GGAGTTGAACTGCTTGATCAGAACAGCAGTATTCGTGTTCCCAGGGAAAAGATGGTTTATATGACTGTCAATGACTCTCAGCCAGAAGATGAAGATTTAAATGTTGCTGAAATCGCTGAC
 321 E  V  Y  M  E  V  I  V  G  E  E  D  A  A  A  A  G  H  A  P  V  H  E  Q  Q  M  D  D  N  E  I  K  T  F  M  P  I  A  W  A
 961 GAAGTTTATATGGAAGTGATCGTAGGAGAGGAGGATGCTGCAGCAGCAGGACACGCGCCGGTGCACGAGCAGCAAATGGATGACAATGAAATCAAAACCTTCATGCCGATTGCATGGGCA
 361 A  A  Y  G  N  N  S  D  G  I  E  N  R  N  G  T  A  S  A  L  L  H  I  D  E  S  A  G  L  G  R  L  A  K  Q  K  P  K  K  R
1081 GCAGCTTATGGTAATAATTCTGATGGAATTGAAAACCGGAATGGCACTGCAAGTGCCCTCTTGCACATAGATGAGTCTGCTGGCCTCGGCAGACTGGCTAAACAAAAACCAAAGAAAAGG
 401 R  R  P  D  S  R  Q  Y  Q  T  A  I  I  I  G  P  D  G  H  P  L  T  V  Y  P  Ⓒ  M  I  Ⓒ  G  K  K  F  K  S  R  G  F  L  K
1201 AGAAGACCTGATTCCAGGCAGTACCAAACAGCAATAATTATTGGCCCTGATGGACATCCTTTGACTGTCTATCCTTGCATGATTTGTGGGAAGAAGTTTAAGTCGAGAGGTTTTTTGAAA
 441 R  H  M  K  N  H  P  E  H  L  A  K  K  K  Y  R  Ⓒ  T  D  Ⓒ  D  Y  T  T  N  K  K  I  S  L  H  N  H  L  E  S  H  K  L  T
1321 AGGCACATGAAAAACCATCCCGAACACCTTGCCAAGAAGAAATACCGCTGTACTGACTGTGATTACACTACCAACAAGAAGATAAGTTTACACAACCACCTGGAGAGCCACAAGCTGACC
 481 S  K  A  E  K  A  I  E  Ⓒ  D  E  Ⓒ  G  K  H  F  S  H  A  G  A  L  F  T  H  K  M  V  H  K  E  K  G  A  N  K  M  H  K  Ⓒ
1441 AGCAAGGCAGAGAAGGCCATTGAATGCGATGAGTGTGGGAAGCATTTCTCTCATGCAGGGGCTTTGTTTACTCACAAAATGGTGCATAAGGAAAAAGGAGCCAACAAAATGCACAAGTGT
 521 K  F  Ⓒ  E  Y  E  T  A  E  Q  G  L  L  N  R  H  L  L  A  V  H  S  K  N  F  P  H  I  Ⓒ  V  E  Ⓒ  G  K  G  F  R  H  P  S
1561 AAATTCTGTGAATACGAGACAGCTGAACAAGGGTTATTGAATCGCCACCTCTTGGCAGTCCACAGCAAGAACTTTCCTCATATTTGTGTGGAGTGTGGTAAGGGTTTTCGTCACCCGTCA
 561 E  L  K  K  H  M  R  I  H  T  G  E  K  P  Y  Q  Ⓒ  Q  Y  Ⓒ  E  Y  R  S  A  D  S  S  N  L  K  T  H  V  K  T  K  H  S  K
1681 GAGCTCAAAAAGCACATGAGAATCCATACTGGGGAGAAGCCGTACCAATGCCAGTACTGCGAATATAGGTCTGCAGACTCTTCTAACTTGAAAACGCATGTCAAAACTAAGCATAGTAAA
 601 E  M  P  F  K  Ⓒ  D  I  Ⓒ  L  L  T  F  S  D  T  K  E  V  Q  Q  H  A  L  I  H  Q  E  S  K  T  H  Q  Ⓒ  L  H  Ⓒ  D  H  K
1801 GAGATGCCATTCAAGTGTGACATTTGTCTTCTGACTTTCTCGGATACCAAAGAGGTGCAGCAACATGCTCTTATCCACCAAGAAAGCAAAACACACCAGTGTTTGCATTGCGACCACAAG
 641 S  S  N  S  S  D  L  K  R  H  I  I  S  V  H  T  K  D  Y  P  H  K  Ⓒ  D  M  Ⓒ  D  K  G  F  H  R  P  S  E  L  K  K  H  V
1921 AGTTCGAACTCAAGTGATTTGAAACGACACATAATTTCAGTTCACACGAAAGACTACCCCCATAAGTGTGACATGTGTGATAAAGGCTTTCACAGGCCTTCAGAACTCAAGAAACACGTG
 681 A  A  H  K  G  K  K  M  H  Q  Ⓒ  R  H  Ⓒ  D  F  K  I  A  D  P  F  V  L  S  R  H  I  L  S  V  H  T  K  D  L  P  F  R  Ⓒ
2041 GCTGCCCACAAGGGCAAAAAAATGCACCAGTGTAGACATTGTGACTTTAAGATTGCAGATCCATTTGTTCTAAGTCGCCATATTCTCTCAGTTCACACAAAGGATCTTCCATTTAGGTGC
 721 K  R  Ⓒ  R  K  G  F  R  Q  Q  S  E  L  K  K  H  M  K  T  H  S  G  R  K  V  Y  Q  Ⓒ  E  Y  Ⓒ  E  Y  S  T  T  D  A  S  G
2161 AAGAGATGTAGAAAGGGATTTAGGCAACAGAGTGAGCTTAAAAAGCATATGAAGACACACAGTGGCAGGAAAGTGTATCAGTGTGAGTACTGTGAGTATAGCACTACAGATGCCTCAGGC
 761 F  K  R  H  V  I  S  I  H  T  K  D  Y  P  H  R  Ⓒ  E  Y  Ⓒ  K  K  G  F  R  R  P  S  E  K  N  Q  H  I  M  R  H  H  K  E
2281 TTTAAACGGCACGTTATTTCCATTCACACGAAAGACTATCCTCACCGGTGTGAGTACTGCAAGAAAGGCTTCCGAAGACCTTCAGAAAAGAACCAGCACATAATGCGACATCATAAAGAA
 801 V  G  L  P  *
2401 GTTGGCCTGCCCTAACAATACTTCTACAGAACGTTTGTAGAGATATTGGCCTTGAAGCAGAAAATTCATTTTAAAGCCAATCAGTCTCATTCACATACAATACTGTATATTGATTTATGC
2521 TGTGTACAAATAGAATTATTACTTCTAGTTGACTTTTTTTTAAATATACATTTTGCTCAGTAGTGTGTTCTGAATTCTATTCAGTTTGTTTAATAAATAGGGAAAACTGGCAACATGCTA
2641 GTTACTTTTAATAAAGTAATCCCTGATTCTATACCGAAGTTTTATATCTTAGAATTTTATATTTATTTAAATATTTACCTTGCTTACCTTGATGGTACTCTTCTAAGACCATTAACTTAA
2761 GGTAACTTTATATTGGTAACTCTGAAAGTATTCATGTTGACTCATTTTTTTCCCCATACATTTCTCACAATAAAATTGTCAGAGACATCTACTAATATAAATGGGAGATTTTACHGTCAG
2881 GTCTAATTATCATAACATGGAAGTCATTTACTTGTCTTGCTTAATATTTTCAGACCACTTGACAGTGAAAGTTTCCATTTGAGCTGTTGCGTCCCTGGCTTTGCTGAGTAAAGAGCAGTG
3001 GCTGGGTTCGTGTTTACTTTTCAAATATACTTCCTTTTCGGCTTTCTTTGGATTATTTACATCTTTTGTCCAGCGTAGCAAACTTTTAGAAAACCTTATTGAAAAACTGTGCTTGCTCAT
3121 GTTGTATTTTGATTATTCTGTCTGTGCGGCTTCATCTTGGAATGGTTGTGTGCTACAAATGACACTTACTGAGGACTGCATTTTGGAATCTCCTAGAGGTAACTCATGGCTTATAGGATC
3241 TTTTGCAACTTTATGTATGTAAATGTACCCTGAATTATATATATACACATATATATCATGTACCTGTGTGTATTGCTTATTTTACATATTTATACACACAACCCCAAGTAGTAGTTGTTT
3361 AAAATCTATAATGAAAAGTATTAAATTTACAATAACATGAAAGATCCAGGGATGCATGAGAGAGCATTTTGTAAGTCATGCTCTTCAGAGAGACTACTCAGGTGAAGAATTAGAAGGAAA
3481 ATAAGGACACTAGTATTTTTAAAGAGTAAAGATATTTTCTTTTAAATATCTTTGGTAATTGAAACATAGAGGTTAAGATGTTTCTAGGTAGAATGTTTTCATACAATTTCACCTCCATGT
3601 CTTTATGTTTTTCTGAAAAGCAAATGAGTATCCAGACATGACTCCCACAGTTCTCTTTGAGAAGCCTGAGAGGGAACTCTGTCTTACCTAGTGAGGGGGATGGAAAAGAAGTGATGGCTC
3721 TGTGGACCAGAGAACGGGTGCTAATTATGACTTCACACTCGGCAAGTTCAGGCTGATCTGTTATTTCTCAGTTACAGTTAGCAAACTTTAAAAACTTAACACTCAAGTTGGCTTTGATTA
3841 AAAGGTAAAGATGTGTTTTAAGTGGATAAGGAAAGTCTGAGGCCTTATTTGGAACATCACTAAGTCTTCCACAAGGTTTTTTGTTTGTTTGTTTTTTTTTTGTTGTTTTTTTTTTCTTAA
3961 GACGGAGTCTTGCTCTGTTGCCCAGGCTGGACTGCAGTGGTGTGATCACTGCAAACCTCTGCAGCTCACTGCAACCTCTGCTTCCTGGGTTCAAGCAATTATCTGCCTCAGCCTCTCGAG
4081 TAGCTGGGATTACAGGCGCCCACCACCACGCCCGGCTAATTTTTTGTATTTTTAATAGAGATGGGGTTTCACCATCTTGGCCAGGCTGGTCTTGAACCCTGACCTCGTGATCCACCTGCC
4201 TCGGCCTTCCATAGTGCTGGGTTTACAGGCGTGAGCCACCGTGCCCAGACACCACATAGGTCTGAATCAGTGTCATACATTCATAAAACAAACTCGGTTAATTAGAACTTGGTTATGTTA
4321 AGACGAATCTGGGAGAACAGAAAACAGTTTTTGGGGTCCCTTCAGTTGGCTATTGGTCCGTATGCATCTAGCACATTGTAGGAGATTTAGAAATTGTCTTCCCACCCGATAGCTGCCTTG
4441 TCACCTCATTATGGTGCTCCATCCCCTGTGTGCTTAGGTTTTTACCTTTCATCTTTCTCTTTGCCATTGATGTTTGTATTCAAGAGTTATATTTTTAGGGTTAGAAATCAAAATATTTGG
4561 TGTTTGGCAAACCTCTGAAGTGCTAGACTGATTTAGTCTAGTTTTAAACCAAGTGCTTTAGGCAGGTGTGAACTCCAGCCCAAATGCCAGTCAAAGTCAAGGCATGGGTTTTCCTAGCCT
4681 ATCTTATAGGAAATTCCTGTACCTTCTTGGCCCCCATAATGTGTTTTTTTTTTTTTTTTTTTTTAAAACTAACTTACAATTTTGTGATCCGTGATTCATTGCCCTGCGATTCTTGAAAGC
4801 TCTGTCTGTTTTTTTGTGAGAACCTTTAAAATCTCCCTTAATTTTTATTTTCCCAGAAATAATGTAAAAACACTTAAATGAAAGTGGAAATGTATTAATTTTAAATCCTATAAAATTAAT
4921 ACAGAAAATATAAATGATTGGGTCATTTAACTATATTTTTTTAAATAAACTGAAAGATAAAGAACACAACACTTCACACATTTTATATTTCTCTTACATACTCCGGAATCATACACAGTT
5041 CTTTTTAAAGCACAACATTAAAACCTTTAAAAGGTATTTAAGGGTTTGGTCAAGTGAATATGATAAAACATACTTGTCTGTATAAAGAGAAAATGAAATTGTAGTCACTGTTATGTACTG
5161 ACATTAGTTACAACCTAGTTTTAATTCTTAAAACATTTTGATTAGCAAAGCTAAAAAAAAATGGATGTTTCAGTTAAATGTTTTAAAGAGGTACAGATTTTTACAAGGACATAATATAAG
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5401 TGTACTTGAGAATAACGTGCAAAAATAAAAATCAGAATATTTTCCTGTTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA 5495
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3′ primer was common to all three cDNAs Bulk cDNA generated by reverse transcription of male or female lymphoblastoid RNA was used as template Fragments predicted from the sequences of cDNA clones 1, 2 and 3 were amplified (Fig 2*b*), confirming the differential splicing shown in Fig 2*a* Some unanticipated fragments were simultaneously amplified (Fig 2*b*)

The ZFX^{804} protein has a primary sequence strikingly similar to those encoded by the mouse *ZFY* homologues (*Zfy*-1 and *Zfy*-2)[3,4], with which it shares virtually identical N and C termini ZFX^{804} and ZFX^{575} proteins are also similar to the human ZFY protein (ref 1, and P B -R and A S -G, unpublished results) Each of these proteins is composed of an acidic domain, a small basic domain, and 13 Cys-Cys/His-His zinc fingers This combination of features is reminiscent of those of eukaryotic transcription activators[6] By analogy, we assume that ZFX and ZFY proteins activate transcription in a sequence-specific fashion When fused to the DNA-binding domain of GAL4, the acidic domains of mouse Zfx and Zfy-2 do activate transcription in yeast[7]

Are the various ZFX and ZFY proteins functionally distinct? All share a small basic domain, which could localize the proteins to the nucleus[8,9] Cys-Cys/His-His zinc-finger domains[10,11] bind to nucleic acids in a sequence-specific fashion The zinc-finger domains of ZFX^{804} and ZFX^{575} are identical and differ from the zinc-finger domain of human ZFY at only 10 of 393 amino acids[2] Thus ZFX^{804}, ZFX^{575} and ZFY proteins could all recognize the same nucleic-acid target sequences, and thereby perhaps regulate transcription of the same genes

The N-terminal portions of the ZFX and ZFY proteins are highly acidic Acidic domains mediate activation in several eukaryotic transcription factors[12-15] The potency of such activating domains may depend more on the number and density of acidic residues than on particular primary sequences[5] In ZFX^{804} and mouse Zfx, Zfy-1 and Zfy-2, the acidic domains are of similar size, each with ~25% of residues acidic By contrast, ZFX^{575} contains an abbreviated acidic domain ZFX^{804} and ZFX^{575} might be expected to have quantitatively different activating potencies Furthermore, acidic domains of transcription factors are believed to interact with other proteins in transcription complexes Truncation of the acidic domain in ZFX^{575} could alter its interactions with other factors, resulting in qualitatively different regulatory properties (Internal ATG codons in human *ZFY* and mouse *Zfx*, *Zfy-1* and *Zfy-2* could allow production of similarly truncated isoforms)

We compared the human *ZFX* cDNA sequence with that of a murine *Zfx* cDNA (ref 7, Fig 3) The coding regions of *ZFX* and *Zfx* show a high degree of similarity It is of interest that the 3′ UTR is also conserved Except for an *Alu* insertion in the human sequence, the entire human 3′ UTR of 3 kb shows over 80% nucleotide identity with the murine 3′ UTR

Given the similarity in the primary structure of the ZFX protein and its Y chromosome-encoded homologues, it is conceivable that the products of the *ZFX* and *ZFY* genes are

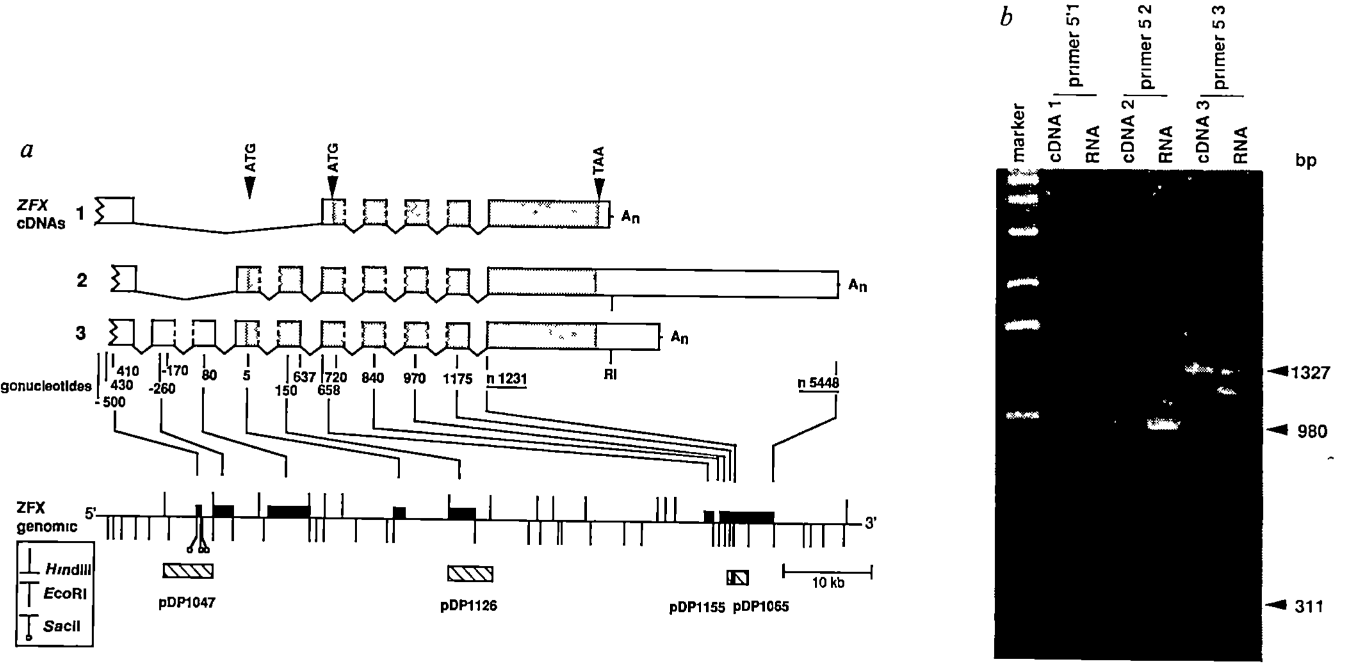
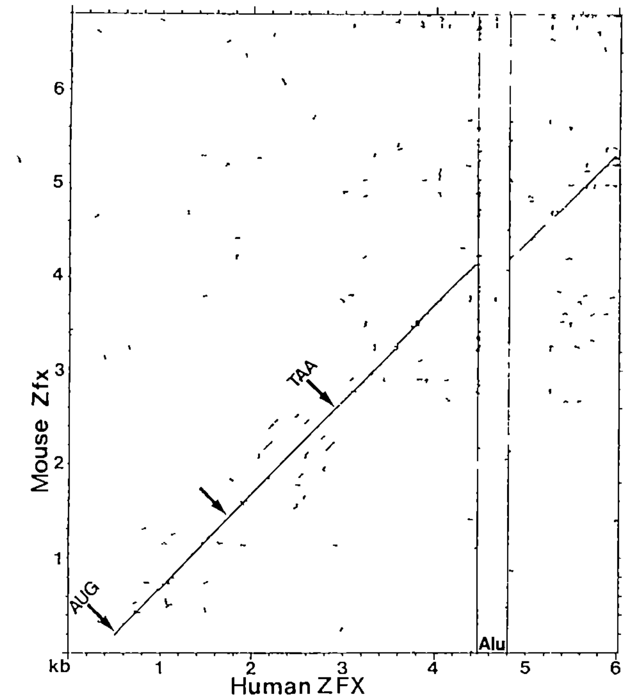


FIG 2 Analysis of alternative ZFX transcripts *a*, Organization of cDNAs and genomic locus Top boxes indicate the minimum number of exons and, with the exception of the 3′-most exon, are not to scale, coding regions are shaded Several splice sites (solid vertical lines of boxes) were defined by DNA sequence comparison among cDNA clones 1, 2 and 3 or by comparison with genomic sequences Additional splices (dashed vertical lines of boxes) are not precisely defined but inferred from comparison of cDNA and genomic restriction maps Boxes bounded by dashed lines may contain more than one exon each Genomic DNA fragments[2] hybridizing (5–10 °C below calculated melting temperature) with *ZFX* oligonucleotides (numbers denote the 11th nucleotide in 20-mer oligonucleotides) are shown by black segments, each contains one or more exons *b*, PCR analysis of differential splicing Three different 5′ primers (20mers) spanning splices unique to cDNAs 1, 2 or 3 have in common the invariant donor site at nucleotide −378, but each includes a unique acceptor (−389 to −378/+647 to +654 (primer 5′1), −383 to −378/−28 to −15 (primer 5′2), −381 to −362 (primer 5′3)) An invariant 3′ primer (+923 to +946) was used Using cDNA clones 1, 2 and 3 as templates, the predicted products were amplified (cDNA 1, 311 base pairs (bp), cDNA 2, 980 bp, cDNA 3, 1,327 bp) Using 'RNA' (bulk cDNA prepared from male lymphoblastoid WHT1659 RNA by reverse transcription) as template, products of the same lengths were obtained In addition, with bulk cDNA, primer 5′2 amplified a 1 1-kb fragment (which may incorporate additional coding exon(s) not detected by cDNA cloning), whereas primer 5′3 amplified a barely detectable 1 4-kb fragment (possibly incorporating additional coding or 5′ UTR exon(s)) and a 1 2-kb fragment (which may lack coding or 5′ UTR exon(s) present in cDNA 3) Controls not shown (1) The 5′ primers are splice specific, yielding products only with the corresponding cDNAs, (2) amplification of cell line RNA, without reverse transcriptase, yielded no products, (3) by using bulk cDNA from female lymphoblastoid cell line WHT1628 as template, products of the same lengths were obtained, and (4) hybridization of Southern blots of these gels verified that the ethidium bromide-detected products originate from *ZFX*

METHODS Poly(A)$^+$ RNA (200 ng) was reverse-transcribed using Moloney reverse transcriptase (Bethesda Research) Forty cycles of amplification were performed using *Taq* polymerase and a DNA Thermo Cycler (Cetus) Each PCR reaction (10%) was separated on an agarose gel and stained with ethidium bromide The marker lane contains a '1-kb ladder' (Bethesda Research)



Homology between human ZFX and mouse Zfx

	% Identity		No of gaps
	DNA	protein	
Coding region			
N-terminal domain	90	92	2
Zinc finger domain	93	99 5	0
3 untranslated region			
5 of *Alu* insertion	85	-	19
3 of *Alu* insertion	81	-	22

FIG 3 Nucleotide and corresponding amino-acid similarity between human *ZFX* (Fig 1) and murine *Zfx* (ref 7) Striking similarity is found not only in the coding region, but also across the entire 3′ UTR (except for an *Alu* insertion, indicated by vertical lines, in the human), and ~20 nucleotides 5′ of the ATG codon Arrows indicate the start codon in *ZFX* cDNAs 2 and 3, the splice site preceding the zinc-finger exon, and the TAA stop codon Numbering (in kb) is with reference to the 5′ ends of the cDNAs The analysis[18] employed a 'window' of 21 and a 'stringency' of 14

functionally interchangeable But the occurrence of isoforms—at least of ZFX—indicates that *ZFX* and *ZFY* could produce transcription factors with qualitatively or quantitatively distinct functions The 3′ UTRs of *ZFX* transcripts are longer and more highly conserved than those found in *Zfy* transcripts[3 4], and could provide a basis for differential post-transcriptional regulation Detailed studies of *ZFX* and *ZFY* transcripts during development should further elucidate their postulated roles in sex determination ☐

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1 Page D C *et al* *Cell* **51**, 1091-1104 (1987)
2 Schneider-Gadicke A *et al* *Cell* **57**, 1247-1258 (1989)
3 Mardon G & Page D C *Cell* **56**, 765-770 (1989)
4 Ashworth A Swift S & Affara N *Nucleic Acids Res* **17**, 2864 (1989)
5 Kozak M *Cell* **44**, 283-292 (1986)
6 Ptashne M *Nature* **335**, 683-689 (1988)
7 Mardon G *et al* *Molec cell Biol* (in the press)
8 Dang C V & Lee W M F *Molec cell Biol* **8**, 4048-4054 (1988)
9 Dingwall C & Laskey R A *A Rev Cell Biol* **2**, 367-390 (1986)
10 Miller J McLachlan A D & Klug A *EMBO J* **4**, 1609-1614 (1985)
11 Brown R S Sander C & Argos P *FEBS Lett* **186**, 271-274 (1985)
12 Ma J & Ptashne M *Cell* **48**, 847-853 (1987)
13 Hope I A & Struhl K *Cell* **46**, 885-894 (1986)
14 Hollenberg S M & Evans R M *Cell* **55**, 899-906 (1988)
15 Godowski P J Picard D & Yamamoto K R *Science* **241**, 812-816 (1988)
16 Schmid C W & Jelinek W R *Science* **216**, 1065-1070 (1982)
17 Fitzgerald M & Shenk T *Cell* **24**, 251-260 (1981)
18 Maizel J V & Lenk R P *Proc natn Acad Sci USA* **78**, 7665-7669 (1981)

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PDGF induction of tyrosine phosphorylation of GTPase activating protein

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THE cascade of biochemical events triggered by growth factors and their receptors is central to understanding normal cell-growth regulation and its subversion in cancer Ras proteins ($p21^{ras}$) have been implicated in signal transduction pathways used by several growth factors, including platelet-derived growth factor (PDGF)[1]. These guanine nucleotide-binding Ras proteins specifically interact with a cellular GTPase-activating protein (GAP)[2] Here we report that in intact quiescent fibroblasts, both AA and BB homodimers of PDGF rapidly induce tyrosine phosphorylation of GAP under conditions in which insulin and basic fibroblast growth factor (bFGF) are ineffective. Although GAP is located predominantly in the cytosol, most tyrosine-phosphorylated GAP is associated with the cell membrane, the site of $p21^{ras}$ biological activity[3,4] These results provide a direct biochemical link between activated PDGF-receptor tyrosine kinases and the $p21^{ras}$-GAP mitogenic signalling system

In human malignancies, *ras* genes are commonly activated as oncogenes and their p21 products are potently mitogenic when microinjected into quiescent fibroblasts[5,6] Yet the function of $p21^{ras}$ proteins in normal mitogenic signal transduction remains unknown It is well established that the GTP-bound forms of $p21^{ras}$ proteins are active and that the GDP-bound forms are inactive[7-9] The GAP protein of relative molecular mass 125,000 ($M_r \sim 125$K) catalyses the conversion of p21-GTP to p21-GDP so that the rate of this recycling is more than 100-fold faster than that intrinsic for p21 (ref 2) GAP has no effect on oncogenic p21 proteins, allowing them to remain in their active GTP-bound states

To investigate whether GAP serves as a substrate for activated growth factor-receptor kinases in intact cells, confluent NIH3T3 cells were rendered quiescent by incubating them overnight in serum-free medium Cells were exposed to saturating concentrations of PDGF BB homodimer for 5 min, after which cell lysates were subjected to immunoblot analysis with an antiphosphotyrosine antiserum (anti-P-Tyr) or anti-GAP peptide serum 638 (ref 10) PDGF BB homodimer induced the specific tyrosine phosphorylation of several proteins (Fig 1*a*) These included the α- and β-PDGF receptors each of M_r 185K (ref 11), a protein of M_r 145K that has been identified as phospholipase C_γ (ref 12), and several other proteins of M_r ranging from 50-125K Anti-GAP peptide serum 638, raised against GAP amino-acid residues 139-152 (ref 10), identified a single band corresponding to a protein of $M_r \sim 125$K, which was not observed in the presence of excess competing peptide (Fig 1*a*, lane 3)

When quiescent cells or cells treated with PDGF BB homodimer were first immunoprecipitated with either polyclonal or monoclonal anti-P-Tyr antibodies followed by immunoblotting with anti-GAP antiserum 638, GAP was detectable only in cells stimulated with PDGF BB homodimer (Fig 1*b*, lane 3) Similar results were observed using anti-GAP peptide serum 677 raised against GAP residues 968-981 (ref 10) Moreover, recovery of GAP by anti-P-Tyr antiserum was specifically inhibited by an excess of competing phosphotyrosine

(Fig 1*b*, lane 4), but not by phosphoserine or phosphothreonine (data not shown) Whereas these experiments were performed using saturating amounts of PDGF BB homodimer (100 ng ml^{-1}), increased levels of anti-P-Tyr-recoverable GAP were also observed using concentrations of PDGF BB that did not maximally induce DNA synthesis (data not shown) Taken together, these results imply that GAP is an early substrate of PDGF-receptor tyrosine kinases or is specifically co-immunoprecipitated with a tyrosine-phosphorylated substrate(s)

When cell lysates were first immunoprecipitated with an antiserum against recombinant GAP, a protein of M_r 125K was detected from either unstimulated (Fig 1*c*, lane 1) or PDGF BB homodimer-stimulated (Fig 1*c*, lane 2) cells Note that we reproducibly observed slightly better recovery of GAP from PDGF-stimulated cell lysates (Fig 1*c*, lane 2) When parallel immunoprecipitates were probed with anti-P-Tyr antiserum, only the 125K GAP band extracted from PDGF-stimulated cells was identified (Fig 1*c*, lane 4) These results confirm that GAP was tyrosine-phosphorylated in response to PDGF BB homodimer The GAP protein contains at least two consensus tyrosine-phosphorylation sites at residues 161 and 720 (ref 13) Recent studies have also indicated that GAP can serve as a substrate of tyrosine kinases *in vitro*[14,15] The precise location of the *in vivo* sites of GAP tyrosine phosphorylation remains to be determined

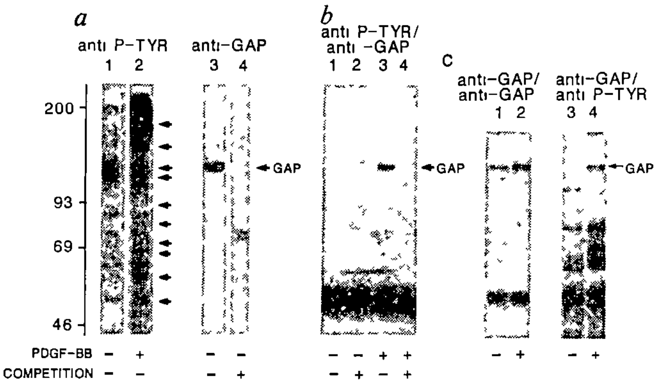

FIG 1 Tyrosine phosphorylation of GAP in PDGF BB homodimer-stimulated NIH3T3 cells *a*, Lanes 1 and 2, immunoblot of total cell lysates (100 μg) using anti-P-Tyr antiserum Cells were either quiescent or treated with PDGF BB homodimer for 5 min as indicated Lanes 3 and 4, immunoblot of cell lysates, using anti-GAP peptide serum 638 (ref 10) in the absence (lane 3) or presence (lane 4) of competing peptide *b*, Immunoblot of anti-P-Tyr antiserum-recovered proteins using anti-GAP antiserum Where noted, phosphotyrosine (5 mM) was added to the lysates before immunoprecipitation for competition Lanes 1 and 2, anti-P-Tyr antiserum immunoprecipitates from quiescent cells, lanes 3 and 4, anti-P-Tyr antiserum immunoprecipitates from PDGF BB homodimer-stimulated cells *c*, Anti-GAP serum 106 immunoprecipitates from quiescent (lanes 1 and 3) and PDGF BB homodimer-stimulated (lanes 2 and 4) cells were analysed by immunoblotting using either anti-GAP peptide serum 638 or anti-P-Tyr antiserum as indicated

METHODS After incubation in serum-free medium for 18 h, confluent NIH3T3 cells were either left untreated or stimulated with PDGF BB homodimer (Amgen) at 100 ng ml^{-1} for 5 min Cultures were rinsed twice in ice-cold PBS containing 1 mM Na_3VO_4, and lysed on ice in 0 5 ml P-Tyr lysis buffer (50 mM HEPES buffer pH 7 5, 1% Triton X-100, 50 mM NaCl, 50 mM NaF, 10 mM sodium pyrophosphate, 5 mM EDTA, 1 mM Na_3VO_4, 1 mM PMSF, in addition to 10 μg ml^{-1} aprotinin, leupeptin, and pepstatin A) Lysates were clarified by centrifugation at 12,000*g* at 4 °C for 10 min, and then immunoprecipitated with affinity-purified anti-P-Tyr antiserum[11,19], monoclonal anti-P-Tyr antibodies (PY 20, PY 69, ICN), or rabbit anti-GAP serum 106 raised against recombinant GAP (residues 128–1,044, J B G, unpublished results) for 2 h on ice Immune complexes were recovered using protein G-agarose (Gammabind G, Genex) Immunoprecipitates were washed five times in P-Tyr lysis buffer and then solubilized in SDS-PAGE sample buffer as previously described[19,29] Aliquots of the whole-cell lysates were removed before immunoprecipitation for immunoblot analysis Gel electrophoresis and immunoblotting were performed as previously described[11,29] Immunoreactive bands were visualized using ^{125}I-labelled protein A followed by autoradiography

We next monitored the recovery of GAP by anti-P-Tyr antiserum at different times after PDGF BB homodimer stimulation of quiescent cells PDGF BB homodimer induced rapid PDGF-receptor autophosphorylation, which increased over the first 10 min and then gradually decreased (Fig 2*a*) Similarly, increased recovery of GAP by anti-P-Tyr was observed within 1 min of PDGF addition, was maximal by $\sim$10 min, and persisted for at least 120 min (Fig 2*b*) In other experiments, we detected increased levels of anti-P-Tyr-recoverable GAP for up to 8 h after PDGF BB homodimer stimulation (data not shown)

GAP has previously been reported to be located in the cell cytosol[2] Because we found that GAP was rapidly tyrosine-phosphorylated in response to PDGF, we performed subcellular fractionation studies to determine the site of GAP tyrosine phosphorylation within the cell In quiescent fibroblasts, GAP was found predominantly in the cystolic fraction (Fig 3*a*, lane 2) But after stimulation of the cells with PDGF BB homodimer for 10 min, we observed a modest but reproducible increase in GAP associated with the particulate fraction concomitant with a slight decrease in the cytosolic component (Fig 3*a*, lanes 3 and 4) Anti-P-Tyr antiserum-recoverable GAP was observed only in the PDGF BB homodimer-stimulated cells and was most abundant in the particulate fraction (Fig 3*b*, lane 3) By comparing the relative signal intensities of anti-GAP immunoblots of anti-P-Tyr-recovered versus total GAP in the respective subcellular fractions, we estimated that at least 15–20% of the membrane-associated GAP protein was recovered by anti-P-Tyr antiserum immunoprecipitation and that <2% of cytosolic GAP could be recovered under the same conditions Because anti-P-Tyr immunoprecipitation is relatively inefficient (C J M , unpublished results), it is probable that a much greater fraction of the particulate (P-100)-localized GAP is tyrosine-phosphorylated in response to PDGF BB homodimer

The specificity of PDGF-induced GAP phosphorylation was analysed by comparing the responses of quiescent NIH3T3 cells to stimulation with saturating concentrations of several growth factors The addition of either PDGF AA homodimer, bFGF, or insulin-induced specific tyrosine phosphorylation of cellular proteins (Fig 4*a*) For example, bFGF caused tyrosine phosphorylation of a previously identified protein of M_r 90K (ref 16), and insulin induced tyrosine phosphorylation of proteins

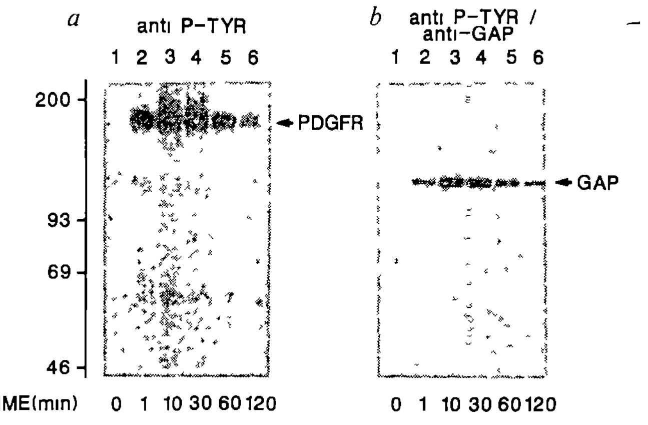

FIG 2 Time course of anti-P-Tyr antiserum recovery of GAP after PDGF BB homodimer stimulation *a*, Anti-P-Tyr antiserum immunoblot analysis of whole-cell lysates from unstimulated cells (lane 1) and cells stimulated by PDGF BB homodimer for the indicated time (lanes 2–6) Arrow denotes the autophosphorylation of PDGF receptors[11] *b*, Anti-GAP peptide serum 677 immunoblot analysis of anti-P-Tyr-antiserum immunoprecipitates from cells either unstimulated (lane 1) or stimulated with PDGF BB homodimer for the indicated times Arrow denotes the immunoreactive GAP protein recovered by anti-P-Tyr antiserum after PDGF BB homodimer stimulation Methods for immunoprecipitation and immunoblotting are described in the legend to Fig 1 Blots were exposed for 15 h using an intensifying screen

with M_rs of 175K and 95K, the latter corresponding to the β-subunit of the insulin receptor[17,18] Stimulation with both PDGF AA and BB homodimers led to increased anti-P-Tyr recovery of GAP (Fig 4*b*, lanes 2 and 3) In other studies using NIH3T3 cells overexpressing epidermal growth factor receptors[19], rapid anti-P-Tyr antiserum recovery of GAP has also been observed after stimulation with epidermal growth factor C J M , unpublished data) By contrast, neither insulin nor bFGF, under conditions in which bFGF was as mitogenically active as the PDGF homodimers, was able to induce detectable GAP tyrosine phosphorylation (Fig 4*b*, lanes 4 and 5) Thus there seem to be differences in the efficiency of GAP tyrosine phosphorylation in response to different growth factors

These studies demonstrate that GAP is tyrosine phosphorylated *in vivo* in response to specific growth factors Recent evidence indicates that PDGF and epidermal growth factor receptor kinases also rapidly induce tyrosine phosphorylation of other proteins, including the c-*raf* gene product[20], phosphatidylinositol kinase[21], and phospholipase C_γ (refs 22-24) The rapid and sustained time course of anti-P-Tyr antiserum recovery of GAP that we observed is very similar to that recently reported for PDGF-stimulated tyrosine phosphorylation of phospholipase C_γ (ref 24) Furthermore, as reported for phospholipase C_γ (ref 24), our preliminary experiments indicate that GAP co-immunoprecipitates with activated PDGF receptors (C J M , unpublished data) Taken together, these findings argue that GAP is an early direct substrate of certain growth factor receptors

Tyrosine phosphorylation is known to modulate the activity of several enzymes, including the *src* gene product[25,26] It is also well established that the $p21^{ras}$ molecule must be located in the membrane to be biologically active[1,3] Thus, our demonstration that GAP recovered by anti-P-Tyr antiserum is associated predominantly with the particulate fraction further indicates that tyrosine phosphorylation may alter the functional interaction of GAP and $p21^{ras}$ In one proposed model, GAP serves as an attenuator of $p21^{ras}$ function[2] If so, tyrosine phosphorylation of GAP could transiently remove its inhibition, allowing the $p21^{ras}$-GTP complex to effect mitogenic signalling through some unknown downstream components Another model proposes that GAP can act as a downstream effector as well as attenuator of $p21^{ras}$ function[2 27 28] Thus, tyrosine phosphorylation of GAP could modulate its effector role, perhaps by coupling the GAP-$p21^{ras}$ complex to other molecules By whatever mechanism tyrosine phosphorylation perturbs GAP-$p21^{ras}$ function, our studies provide direct biochemical evidence linking these important growth regulatory molecules to the mitogenic signalling cascade activated by growth factors ☐

Note added in proof We have also observed tyrosine phosphorylation of GAP in NIH3T3 cells transformed by oncogenes encoding non-membrane-spanning as well as membrane-spanning tyrosine kinases

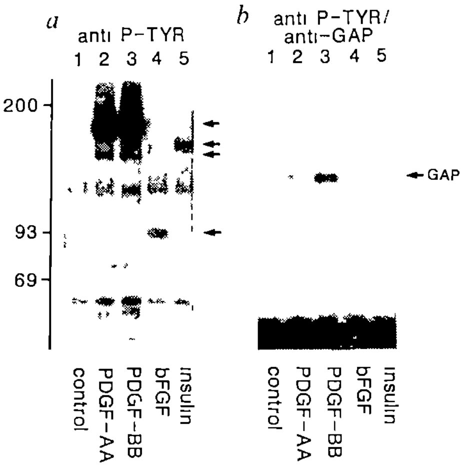

FIG 4 Anti-P-Tyr antiserum recovery of GAP in response to different growth factors *a*, Anti-P-Tyr antiserum immunoblot analysis of whole-cell lysates from unstimulated (lane 1) cells or cells treated for 10 min with PDGF AA homodimer (100 ng ml^{-1}, lane 2), PDGF BB homodimer (100 ng ml^{-1}, lane 3) basic FGF (200 ng ml^{-1}, lane 4), or insulin (100 μg ml^{-1}, lane 5) Tyrosine-phosphorylated bands induced by different growth factors are indicated by arrows *b*, Immunoblot of anti-P-Tyr antiserum-recovered proteins using anti-GAP peptide serum Anti-P-Tyr antiserum immunoprecipitates were prepared from cells treated as in *a* Methods are as described in Fig 1 Blots were exposed to film for 4 days

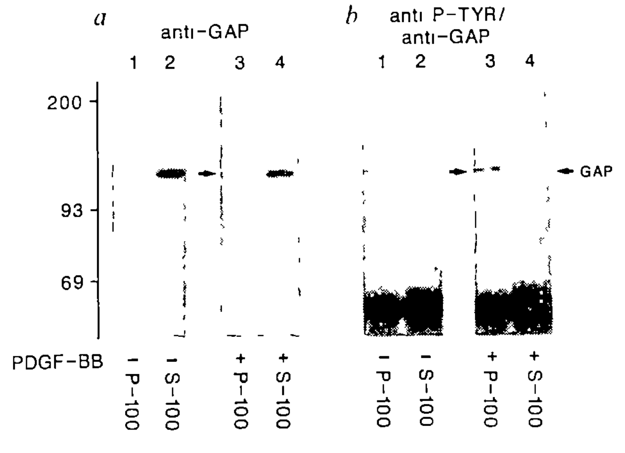

FIG 3 Subcellular localization of GAP following PDGF BB homodimer stimulation *a*, Anti-GAP peptide serum 638 immunoblot of protein extracts (40 μg per lane) from either particulate (P-100) or cytosolic (S-100) cell fractions Cells were either quiescent (lanes 1 and 2) or stimulated with PDGF BB homodimer for 10 min (lanes 3 and 4) *b*, Anti-GAP peptide serum 638 immunoblot of anti-P-Tyr antiserum-recovered proteins from subcellular fractions of unstimulated (lanes 1 and 2) and PDGF BB homodimer-stimulated (lanes 3 and 4) cells Blots were exposed to film for 5 days

METHODS After incubation in serum-free medium for 18 h, confluent NIH3T3 cells (3 × 100-mm plates) were either left untreated or stimulated with PDGF BB homodimer at 100 ng ml^{-1} for 10 min Cultures were then rinsed twice with cold PBS containing 1 mM Na_3VO_4, and then 0 75 ml hypotonic lysis buffer (20 mM HEPES buffer pH 7 4, 5 mM sodium pyrophosphate, 5 mM EGTA, 1 mM $MgCl_2$, 1 mM Na_3VO_4, 1 mM PMSF, in addition to 10 μg ml^{-1} aprotinin, leupeptin, and pepstatin) was added to each plate on ice Cells were collected by scraping into a Dounce homogenizer with a tight-fitting pestle and subsequently lysed with 40 strokes After centrifugation at 1000*g* for 5 min, the supernatant fraction was ultracentrifuged at 100,000*g* for 45 min The cytosolic (S-100) supernatant fraction was removed, and the insoluble pellet was resuspended and recentrifuged The resulting pellet (particulate fraction) was then solubilized in 1 ml P-Tyr lysis buffer (see legend to Fig 1) Cytosolic fractions were concentrated to 1 ml by ultrafiltration using Centricon-10 filters (Amicon) Under these conditions, cell protein recovery in P-100 and S-100 fractions were about equal Aliquots (40 μg) from each fraction were used for anti-GAP peptide serum immunoblot analysis For phosphotyrosine analysis, aliquots of each fraction containing 400 μg protein were first immunoprecipitated with anti-P-Tyr antiserum followed by immunoblotting with anti-GAP peptide serum as described in Fig 1 legend

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1 Mulcahy L S Smith M R & Stacey D W *Nature* **313**, 241-243 (1985)
2 Trahey M & McCormick F *Science* **238**, 542 545 (1987)
3 Willingham M C Pastor I Shih T Y & Scolnick E M *Cell* **19**, 1005-1014 (1980)
4 Willumsen B M *et al Nature* **310**, 583 586 (1984)
5 Stacey D W & Kung H F *Nature* **310**, 508 511 (1984)
6 Feramisco J R *et al Cell* **38**, 109-117 (1984)
7 DeVendittis E Vitelli A Zahn R & Fasano O *EMBO J* **5**, 3657 3663 (1986)
8 Marshall M S Gibbs J B Scolnick E M & Sigal I S *Molec cell Biol* **7**, 2309-2315 (1987)
9 Gibbs J B *et al J biol Chem* **262**, 10426 10429 (1987)
10 Marshall M S *et al EMBO J* **8**, 1105-1110 (1989)
11 Matsui T *et al Science* **243**, 800-804 (1989)
12 Wahl M I *et al Proc natn Acad Sci USA* **86**, 1568-1572 (1989)
13 Vogel U S *et al Nature* **335**, 90-93 (1988)
14 Gibbs J B *et al* in *The Guanine-Nucleotide Binding Proteins Common Structural and Functional Properties* (eds Bosch L Kraal B & Parmeggiani A) 191-200 (Plenum New York 1989)
15 Gibbs J B *et al* in *ADP Ribosylating Toxins and G Proteins Insights into Signal Transduction* (eds Moss J & Vaughn M) (American Society of Microbiology Washington DC) (in the press)
16 Coughlin S R *et al J biol Chem* **263**, 988-993 (1988)
17 Pang D T *et al J biol Chem* **260**, 7131-7136 (1985)

18 Rosen O M *Science* **237**, 1452-1458 (1987)
19 DiFiore P P *et al* *Cell* **51**, 1063-1979 (1987)
20 Morrison D K Kaplan D R Rapp U & Roberts T M *Proc natn Acad Sci USA* **85**, 8855-8859 (1988)
21 Kaplan D R *et al* *Cell* **50**, 1021-1029 (1987)
22 Wahl M I *et al* *Molec cell Biol* **9**, 2934-2943 (1989)
23 Margolis B *et al* *Cell* **57**, 1101-1107 (1989)
24 Meisenhelder J Suh P G Rhee S G & Hunter T *Cell* **57**, 1109 1122 (1989)
25 Hunter T & Cooper J A *A Rev Biochem* **54**, 897-930 (1985)
26 Piwnica Worms H *et al* *Cell* **49**, 75-82 (1987)
27 Cales C Hancock J F Marshall C J & Hall A *Nature* **332**, 548-550 (1988)
28 McCormick F *Cell* **56**, 5 8 (1989)
29 DiMarco E *et al* *Oncogene* **4**, 831-838 (1989)

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Sequence-specific RNA binding by the HIV-1 Rev protein

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THE human immunodeficiency virus type 1 (HIV-1) Rev protein acts post-transcriptionally to increase the amounts of the viral *gag–pol* and *env* messenger RNAs in the cytoplasm of infected cells[1 2]. The mechanism of Rev action is uncertain. Possibilities include an accelerating effect on the rate of export of its mRNA targets from the nucleus[3–6] and/or modulation of the splicing of pre-mRNAs (reviewed in ref 7) Both the *gag–pol* and *env* mRNAs contain a sequence that is required for responsiveness to Rev—the Rev responsive element, RRE[3,4,6,8]. Here we show that Rev is a sequence-specific binding protein, whose binding site is the RRE. This information should help to clarify the mechanism by which Rev acts.

RNA binding experiments were performed using extracts or fractions containing or lacking Rev expressed in *Escherichia coli* from plasmid pZ76-Rev This plasmid was constructed by inserting a *rev* complementary DNA clone into the the expression vector plasmid pRW76, introduced into *E coli* strain XA-90, and *rev* expression induced by treatment with isopropylthiogalactoside (IPTG) In XA-90 (pZ76-Rev) cells, IPTG specifically induced the expression of a polypeptide of relative molecular mass 18,000 (M_r 18K), which was not observed after IPTG treatment of non-transformed XA-90 cells (Fig 1*a*) An immunoblot analysis using a polyclonal α-Rev antibody confirmed that the IPTG-induced 18K polypeptide was Rev (Fig 1*b*)

An RNase protection-mobility shift assay was designed to test whether Rev binds to RNA The RNA binding substrate was an SP6-derived ^{32}P-labelled RNA containing sequences from the HIV-1 *Bgl*II-*Bam*HI fragment (positions 7,201-7,721, ref 9), which encompasses the RRE (Fig 2, bottom, pGEM-HIV1 RRE) After incubation of the ^{32}P-labelled RNA substrate in the *E coli* extracts, the reaction mixtures were extensively treated with RNases T1 and *A*, and ^{32}P-labelled RNA-protein complexes resolved on a native polyacrylamide-agarose gel

A protein in the Rev-containing extract bound to the RRE-containing ^{32}P-labelled RNA substrate (Fig 2) An RNAse-resistant ^{32}P-labelled RNA fragment of retarded electrophoretic mobility was present in the extract prepared from IPTG-treated XA-90 (p76-Rev) cells (lane 4), but not in extracts prepared from non-induced cells (lane 3), or extracts prepared from IPTG-treated XA-90 cells (lane 2) A partially purified Rev fraction (Fig 1*b*, DEAE, 0 2) also supported formation of the ^{32}P-labelled RNA complex (lane 6), whereas complex formation was not detected in a DEAE fraction that lacked Rev (Fig 1*b*, DEAE, 0 4, Fig 2, lane 5) The ^{32}P-labelled RNA complex was eliminated by addition of SDS (lane 7) or proteinase K (lane 8), indicating that it contained both RNA and protein Finally, the ^{32}P-labelled RNA-protein complex was not formed if th RNase was added before rather than after the addition of th Rev-containing extract (lane 8) Taken together these result indicate that a protein binds to the RRE-containing RNA sub strate and protects a region of it from RNase digestion

Formation of the RNA-protein complex is specific to a RRE-containing substrate An unrelated adenovirus RNA sub strate (MINX, lane 11), or an HIV-1 RNA substrate that con tains sequences upstream of the RRE (580, lane 10), did no

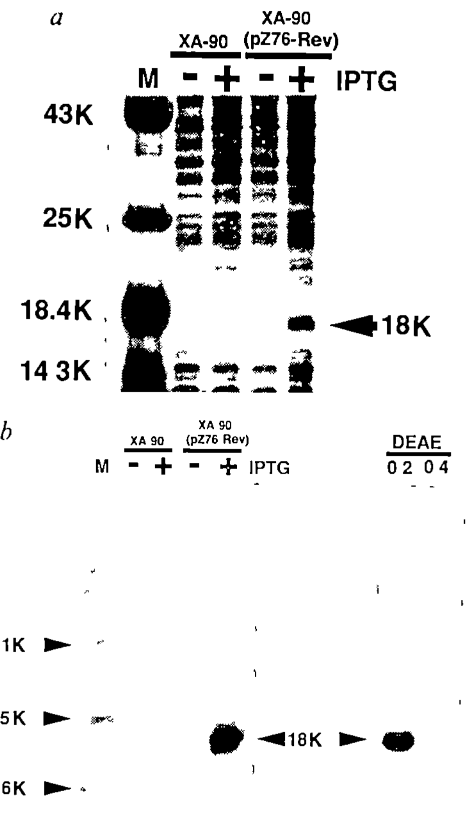
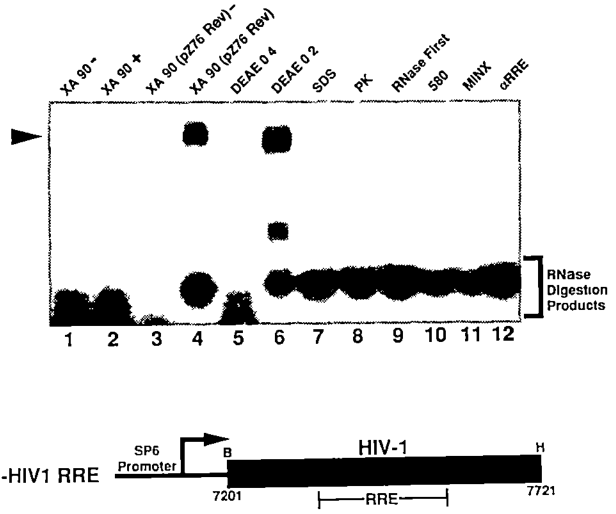


FIG 1 Detection of Rev synthesized in *E coli* *a*, Coomassie blue-stained 12 5% SDS-polyacrylamide gel of protein (~35 μg) from the various extracts Extracts were prepared from either XA-90 or XA-90 (pZ76-Rev) cells with or without IPTG treatment as indicated Migration of protein M_r standards is shown on the left The arrow denotes the IPTG-induced polypeptide in XA-90 (pZ76-Rev) cells of M_r ~18K *b*, Immunoblot analysis Protein (~3 5 μg) from the various extracts was fractionated on a 12 5% SDS-polyacrylamide gel, and Rev detected by immunoblotting using a polyclonal α-Rev antibody The unfractionated extracts (left) were identical to those used in *a* Chromatographic fractions from a DEAE-Sephacryl column loaded with an IPTG-induced XA-90 (pZ76-Rev) extract are shown on the right Migration of ^{14}C-labelled M_r standards (M) is shown on the left

METHODS *a*, Plasmid pZ76-Rev was constructed by inserting the *Eco*NI–*Hin*dIII fragment from the plasmid pcREV (ref 17), which contains a *rev* cDNA clone, between the *Nco*I and *Hin*dIII sites of an *E coli* expression vector plasmid pRW76 (pRW76 is a derivative of the plasmid pLPK76-7, ref 18) The *Eco*NI site (position 6,005) is within the *rev* cDNA ATG translation initiation codon and was joined to the *Nco*I site of pRW76 to restore the translation initiation site and reading frame IPTG-induction and preparation of sonicated *E coli* extracts is described in ref 19 *b*, The α-Rev antibody was prepared by immunizing rabbits with an affinity-purified *E coli*-derived function protein containing Rev sequences encoded by plasmid pSVSX1 (ref 4) The α-Rev antibody (1 1,500 dilution) was incubated with the nitrocellulose for 1 h at 4 °C Antibody–antigen complexes were detected using ^{125}I-labelled protein A (NEN) and autoradiography DEAE fractions were prepared by loading an IPTG-treated XA-90 (pZ76-Rev) extract onto a DEAE-Sephacryl column in buffer A (ref 19) in 50 mM NaCl After collection of a flow-through fraction, the column was eluted with steps of 100 mM, 200 mM, 400 mM and 1 M NaCl Although all fractions contained significant amounts of total protein, immunodetectable Rev was present only in the 200 mM fraction pport formation of the RNA-protein complex Likewise, the rotein also did not bind to the antisense transcript of plasmid GEM-HIV1 RRE (α-RRE, lane 12)

To verify that the RNA-binding protein was in fact Rev, three dditional experiments were performed (Fig 3) First, the size f the RNA-binding protein was determined The proteins in a ev-containing extract were separated by preparative SDS-AGE The gel lane was divided into eight slices, the proteins luted from each slice, the SDS removed by acetone precipitation, and the precipitated proteins resuspended in 6 M guanidine ydrochloride to denature the polypeptide chains[10,11] After moving the guanidine hydrochloride by dialysis, each fraction as assayed for RNA-binding activity All of the RNA-binding ctivity was in the region of the SDS-polyacrylamide gel corresponding to an M_r of 18-20K (Fig 3*a*), the only fraction that ontained Rev (Fig 1*b*, and data not shown)

Next, we asked whether Rev was physically associated with he ^{32}P-labelled RNA substrate If so, an α-Rev antibody should bind to the ^{32}P-labelled RNA-protein complex and alter its electrophoretic mobility ^{32}P-labelled RNA-protein complexes were formed as described above Before loading the complexes on the gel, either the α-Rev antibody or a control pre-immune serum was added to the reaction mixtures (Fig 3*b*) As the amounts of the polyclonal α-Rev antibody added increased, the band corresponding to the normal ^{32}P-labelled RNA-protein complex diminished and concomitantly several bands of reduced electrophoretic mobility appeared By contrast, the addition of comparable amounts of a control pre-immune serum did not affect either the amount or the electrophoretic mobility of the normal ^{32}P-labelled RNA-protein complex



IG 2 Analysis of RNA binding by an RNase protection–mobility shift assay he standard RNA-binding reaction contained the plasmid pGEM-HIV1 RRE ^{2}P-labelled RNA substrate and extract prepared from IPTG-treated XA-90 pZ76-Rev) cells (lane 4) The main variation from this standard reaction is ndicated above each lane Lanes 1–9, plasmid pGEM-HIV1 RRE ^{32}P-labelled NA substrate Lanes 4 and 7–12, extract prepared from IPTG-treated XA-90 pZ76-Rev) cells Lanes 1 and 2, extract prepared from non-induced and PTG-induced XA-90 cells, respectively Lane 3, extract prepared from non-nduced XA-90 (pZ76-Rev) cells Lanes 5 and 6, DEAE (0 4 M) and 0 2-M ractions of an IPTG-treated XA-90 (pZ76-Rev) cell extract, respectively anes 7 and 8, identical to lane 4 except that 0 1% SDS or 0 4 μg proteinase , respectively, were added to the sample before loading on the gel Lane 9, similar to lane 4 except that the ^{32}P-labelled RNA substrates were ncubated with the RNases before addition of the extract Lane 10, HIV-1 580 RNA substrate Lane 11, adenovirus MINX RNA substrate[20] Lane 12, antisense transcript of plasmid pGEM-HIV1 RRE The position of the RNA–protein complex is indicated by the arrowhead The structure of the ^{32}P-labelled RNA probe run-off at the *Hin*dIII site (position 7,721) is shown below the autoradiograph Black box, HIV-1 sequences, B, *Bgl*II site, H, *Hin*dIII site The minimal HIV-1 RRE (positions 7,361–7,569) as defined in ref 3 is indicated

METHODS ^{32}P-labelled RNA probes were synthesized by *in vitro* transcription using SP6 or T7 RNA polymerase and full-length transcripts purified on a 5% denaturing polyacrylamide gel RNA binding reactions contained 10–20 μl extract or fraction, 2 μl ^{32}P-labelled RNA probe (36,000 Cerenkov c p m), and buffer A (ref 19) in a final volume of 25 μl Binding reactions were at 30 °C for 15 min, after which 2 5 U RNase T1 (Pharmacia) and 1 25 μg RNase A (Pharmacia) were added and incubation continued for 10 min The reaction mixtures were directly loaded onto a native 6% polyacrylamide (60 1 cross-linking ratio)–0 5% agarose gel The electrophoresis buffer was 100 mM Tris-HCl, pH 8 8, 50 mM glycine The 580 RNA substrate was synthesized from a plasmid in which the 580-nucleotide *Bgl*II–*Bgl*II HIV-1 DNA fragment (positions 6,621–7,200) was cloned into the *Bam*HI site of the plasmid pGEM3 (Promega) polylinker in the orientation such that the sense strand was transcribed using SP6 RNA polymerase

Finally, we investigated whether Rev could be directly detected in the RNA-protein complex The RNA-protein complex was resolved on a native gel, the band excised, the proteins resolved on a SDS-polyacrylamide gel, and Rev detected by immunoblot analysis The immunoblot (Fig 3*c*) confirmed that the 18K Rev protein was indeed present in the RNA-protein complex

These experiments show that Rev binds specifically to an RRE-containing RNA substrate To demonstrate that Rev bound to the RRE, various derivatives of the RNA substrate were analysed (Fig 4) The 208-nucleotide *Sty*I-*Sau*3AI fragment (positions 7,361–7,569) is the minimal sequence that enables a viral RNA to be affected by Rev (ref 3) Figure 4*a* shows the results of RNA binding experiments using 3′ deletion derivatives of plasmid pGEM3-HIV1 RRE Rev bound equally well to an RNA whose 3′ end was at the *Sau*3AI site (position 7,569) as it did to the original substrate whose 3′ end is at the *Hin*dIII site (position 7,721) But Rev did not bind to an RNA whose 3′ end was at the *Hin*fI site (position 7,540) or the *Alw*NI site (position 7,491) Thus, the 3′ boundary of the region required for Rev binding is within the RRE, between positions 7,540 and 7,569

To determine the 5′ boundary of the Rev-binding site, a comparable 5′ deletion analysis was performed (Fig 4*b*) Rev bound equally well to an RNA whose 5′ end was at the *Mbo*II site (position 7,312) as it did to the original substrate whose 5′ end was at the *Bgl*II site (position 7,201) But further 5′ deletion within the minimal RRE to the *Hae*III site (position 7,424) or *Alw*NI site (position 7,491) eliminated Rev binding Thus, the 5′ boundary of the region required for Rev binding is within the RRE, between positions 7,312 and 7,424

Our *in vitro* binding data is in excellent agreement with the results of a previous *in vivo* study[3], which defined a minimal RRE based on Rev function Deletions that abolish RRE function also prevent Rev binding Therefore the minimal RRE apparently corresponds to the minimal Rev-binding site The RNA region required for Rev binding is relatively large (minimally 116 nucleotides, from the *Hin*fI site to the *Hae*III site) Perhaps Rev binding requires an RNA structure that is formed by two or more non-contiguous RNA regions In fact, the RRE is within an RNA region predicted to have extensive secondary structure[3]

Relatively few sequence-specific RNA-binding proteins have been analysed and only one protein motif, the ribonucleoprotein (RNP) consensus sequence (reviewed in ref 12), has been shown to be involved in sequence-specific RNA binding[13] Rev does not contain an RNP consensus, indicating that its RNA-binding motif could be of a novel class The N-terminal portion of Rev is rich in arginine residues and mutations in this region can interfere with Rev function[14] It is tempting to speculate that this arginine-rich basic region contributes to the RNA-binding activity of Rev

Besides this arginine-rich region, Rev contains at least one other functional domain located near the C terminus[14] Our results imply that one of the functional domains of Rev corresponds to the RNA-binding region (presumably the basic region) The other functional domain(s) presumably provides an effector function, which could interact with a cellular com-

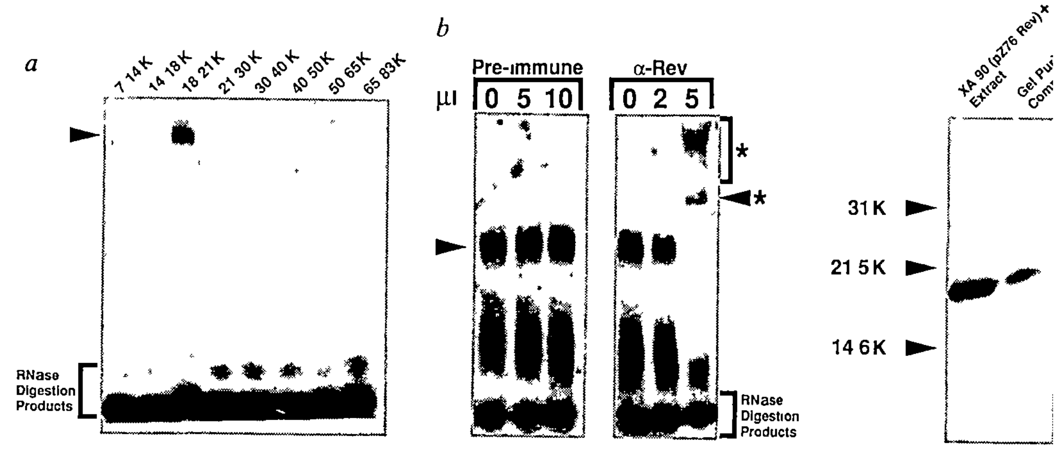


FIG 3 Rev is the RNA-binding protein *a*, Guanidine renaturation of RNA-binding activity Each RNA-binding reaction contained 20 μl of the gel-purified protein fraction indicated above the lane METHODS Extract (2 3 mg) prepared from IPTG-treated XA-90 (pZ76-Rev) cells was fractionated on a preparative 12 5% SDS-polyacrylamide gel The gel lane was divided into eight slices, each slice was diced using a razor blade, and soaked overnight at room temperature in 1 ml elution buffer[10] Polyacrylamide was removed by centrifugation, proteins precipitated by addition of five volumes of 100% acetone and incubation on dry ice for 3 h Precipitated proteins were collected by centrifugation, rinsed in 80% acetone-20% elution buffer without SDS, resuspended in 50 μl of 6 M guanidine hydrochloride-0 1% Nonidet P-40-0 1 M KCl and incubated overnight at room temperature Samples were extensively dialysed against buffer A (ref 19) *b*, An α-Rev antibody alters the electrophoretic mobility of the ^{32}P-labelled RNA-protein complex The type and amount (μl) of antiserum added to the reaction mixtures is indicated above each lane The position of the normal RNA-protein complex is indicated by the arrowhead on the left The positions of RNA-protein complexes of altered electrophoret mobility are indicated by the starred arrowhead and brackets on the rigt The faster-migrating complex is due to partial proteolysis of Rev in th particular extract *c*, Rev is present in the ^{32}P-labelled RNA-protein comple The ^{32}P-labelled RNA-protein complex resolved on a native gel was locate by autoradiography, the gel slice excised, and directly inserted into a lar of a 12 5% SDS-polyacrylamide gel Rev was detected by immunobl analysis using the α-Rev antibody as described in the legend to Fig 1*b*

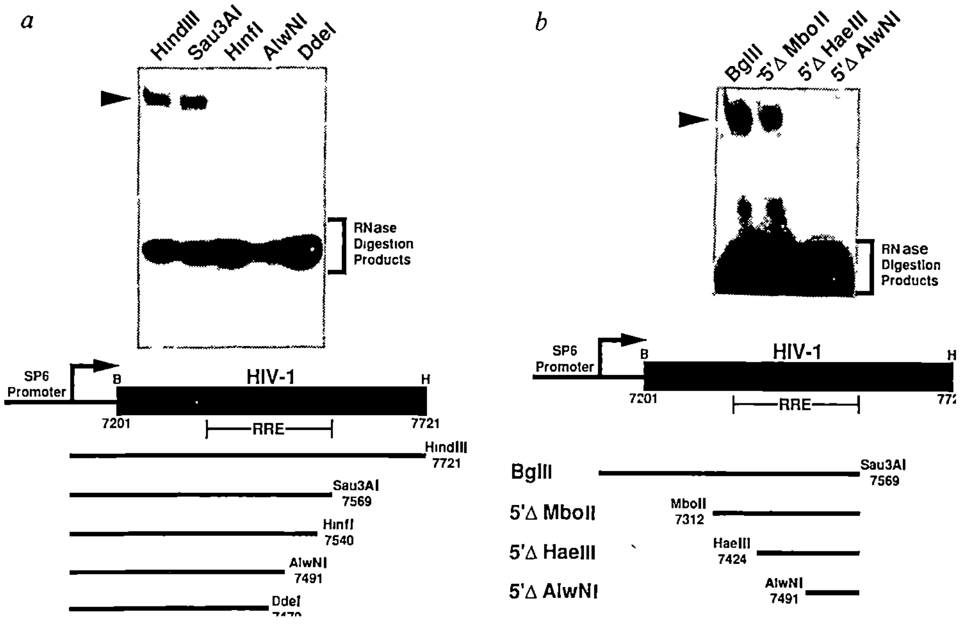


FIG 4 Delineation of the Rev-binding site *a*, 3′ deletion analysis Truncated ^{32}P-labelled RNA substrates were synthesized from the plasmid pGEM-HIV1 RRE DNA templates cleaved at the indicated restriction sites Each RNA binding reaction contained an identical amount of ^{32}P-labelled RNA substrate A schematic diagram of the ^{32}P-labelled RNA substrates is shown below the autoradiograph *b*, 5′ deletion analysis Each RNA-binding reaction contained an identical amount of ^{32}P-labelled RNA substrate A schematic diagram of the ^{32}P-labelled RNA substrates is shown below the autoradiograph
METHODS 5′ deletion constructs were prepared by inserting either the *Mbo*II-*Hin*dIII (positions 7,312-7,721), *Hae*III-*Hin*dIII (positions 7,424-7,721), or *Alw*NI-*Hin*dIII (positions 7,491-7,721) HIV-1 DNA fragments between the *Hin*cII and *Hin*dIII sites of plasmid pGEM4 (Promega) SP6-transcription templates were prepared by cleaving these constructs with *Sau*3AI

ponent involved, for example, in mRNA nuclear export or pre-mRNA splicing Some mutations within this C-terminal domain have a dominant-negative phenotype[14] A Rev protein that contains an RNA-binding domain but lacks the putative effector function would compete with wild-type Rev for binding to the RRE and thus have a dominant-negative phenotype

The HTLV1 Rex protein seems to function similarly to Rev (see refs 15, 16) It is therefore likely that Rex is also a sequence-specific RNA-binding protein Rex can functionally substitute for Rev in co-transfection experiments[15] This raises the possibility that Rev and Rex bind to the same RNA site even though they have no detectable amino-acid similarity ☐

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1 Feinberg M B Jarrett R F Aldovini A Gallo R C & Wong-Staal F *Cell* **46**, 807-817 (1986)
2 Sodroski J *et al* *Nature* **321**, 412-417 (1986)
3 Malim M H Hauber J Le S Y Maizel J V & Cullen B R *Nature* **338**, 254-257 (1989)
4 Hammarskjold M-L *et al* *J Virol* **63**, 1959-1966 (1989)
5 Felber B K Hadzopoulou Cladaras M Cladaras C Copeland T & Pavlakis G N *Proc. nat* *Acad Sci USA* **86**, 1495-1499 (1989)
6 Emerman M Vazeux R & Peden K *Cell* **57**, 1155-1165 (1989)
7 Green M R & Zapp M L *Nature* **338**, 200-201 (1989)
8 Hadzopoulou Cladaras M *et al* *J Virol* **63** 1265-1274 (1989)
9 Ratner L *et al* *Nature* **313** 277-284 (1985)
10 Hager D A & Burgess R R *Analyt Biochem* **109**, 79-86 (1980)
11 Briggs M R Kadonaga J T Bell S P & Tjian R *Science* **234**, 47-51 (1986)
12 Bandziulis R J Swanson M S & Dreyfuss G *Genes Dev* **3**, 431-437 (1989)
13 Query C C Bentley R C & Keene J D *Cell* **57** 89-101 (1989)
14 Malim M H Bohnlein S Hauber J & Cullen B R *Cell* **58**, 205-214 (1989)
15 Rimsky, L *et al* *Nature* **335**, 738-740 (1988)
16 Seiki M Inoue J I Hidaka M & Yoshida, M *Proc. natn Acad Sci USA* **85**, 7124-7128 (1988)
17 Malim M H Hauber J Fenrick R & Cullen B R *Nature* **335**, 181-183 (1988)
18 Keegan L Gill G & Ptashne M *Science* **231**, 699-704 (1986)
19 Lin Y S Carey M F Ptashne M & Green M R *Cell* **54**, 659-664 (1988)
20 Zillmann M Rose S D & Berget S M *Molec cell Biol* **7**, 2877-2883 (1987)

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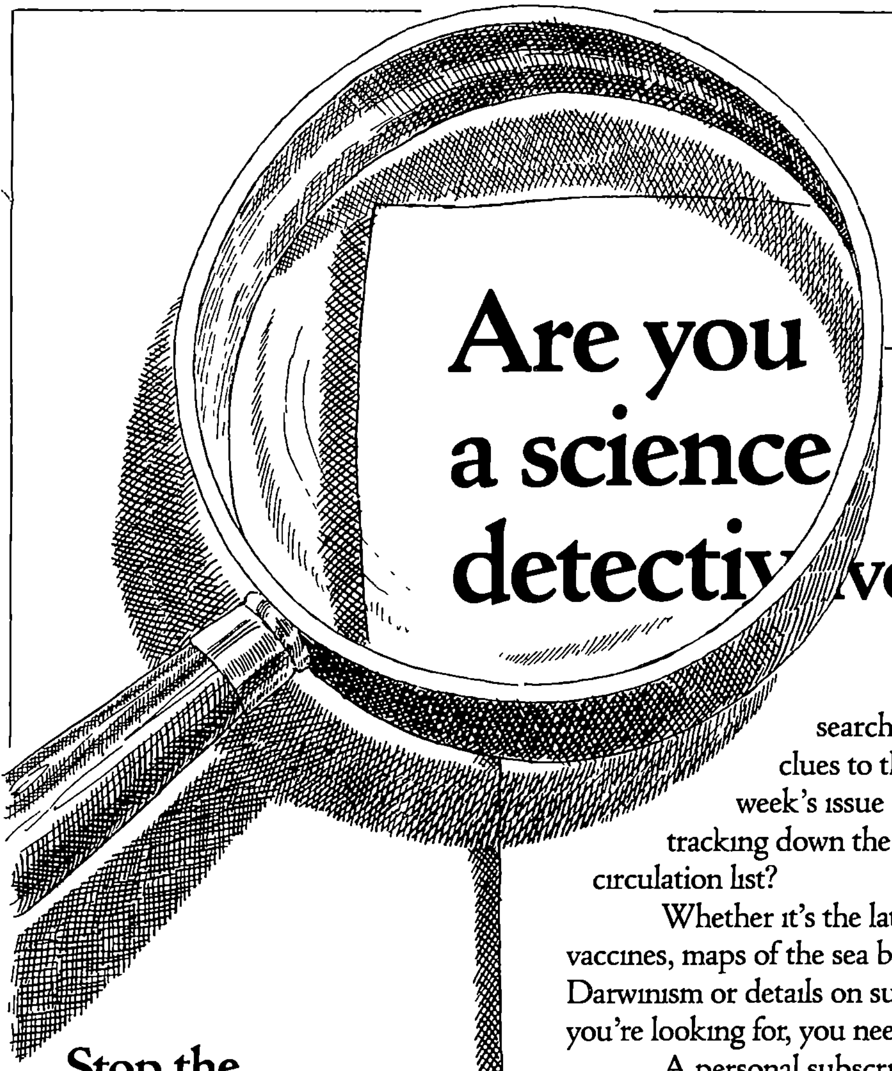

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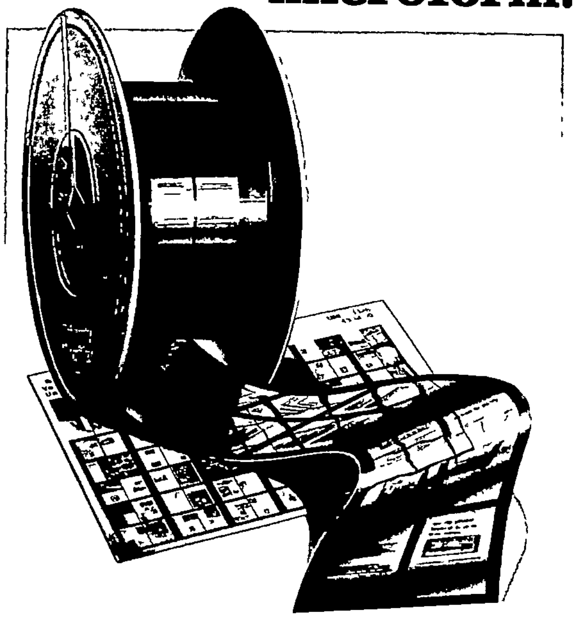

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Pre-Christmas product selection

A gel elutor for extracting nucleic acids or protein, a cryopreservation system for freezing/thawing mouse embryos and a neurotoxicological evaluation service based on the leech brain are just a few of the products outlined below.

NEW from Bioproducts for Science, Inc. is a **mouse anti-rat natural killer cell monoclonal antibody** that recognizes and reacts with all rat NK cells, large granular lymphocytes and neutrophils (*Reader Service No. 100*). The mouse anti-rat NK cell mAb has applications in the study of transplant immunology and in studies requiring *in vivo* depletion of NK cells. The mAb is available in three forms: 5-ml vials of culture supernatant with 5 μg/ml of antibody; 0.25-ml vials of ascites with 1–2 mg/ml of antibody; and as a $F(ab')_2$ FITC conjugate in 1-ml vials containing 700 μg/ml of antibody. All products are supplied with a procedures guide.

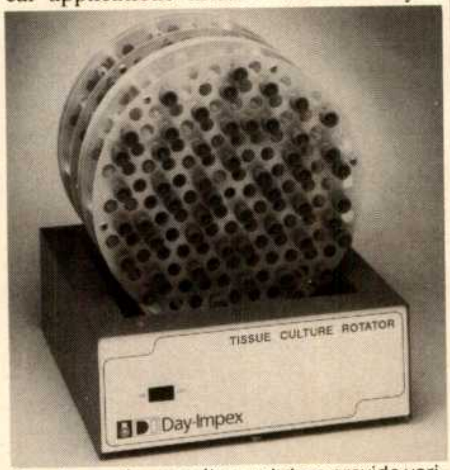
Handy helpers

According to Fisher Scientific, the Fisher Biotech Gel Eluter can simultaneously extract samples from up to six gel slices using a **vertical gel elution technique** (*Reader Service No. 101*). The vertical design of the apparatus is said to offer advantages over conventional horizontal elution methods by accelerating migration of the sample, minimizing electroconvection and reducing build-up around the seive pores. The elution column is divided by a porous frit into a sample chamber and removable recovery chamber. The frit acts as a physical barrier to the gel slice while allowing the eluted material to pass into the recovery chamber, to be drawn off by syringe. Fisher says, the eluter will recover virtually any nucleic acid or protein sample with a 75–100 per cent recovery efficiency. The apparatus will work with most power supplies and each elution column requires a current of 1–2 mA.

FisherBiotech Gel Eluter: designed for vertical gel elution.

The $295 (US) gel eluter can also be used as a tube gel isoelectric focusing apparatus. It can run up to 12 tube gels under identical conditions used in IEF analysis or as the first dimension separation in 2-D electrophoresis.

For the smooth, continuous **rotation of bottles and test tubes**, Day-Impex has introduced a new line of Tissue Culture Rotators (*Reader Service No. 102*). Typical applications include the monolayer culture of anchorage-dependent cells for increased production of, for example, monoclonal antibodies, viruses, enzymes and other secreted products. Four models of the bottle rotator are available which hold between two and ten bottles and operate at speeds of 0.5–8 r.p.m. A tachometer on the control panel provides a readout of rotational speed. The tissue culture test tube rotators are designed to operate in incubators up to temperatures of 60 °C. A single-drum rotator has a capacity of 84 25-mm tubes or 150 16-mm tubes and can operate at rotational speeds of up to 70 r.p.m. The tube capacity can be doubled by the addition of an extra drum. The angle of the drum is adjustable between 5° and 90° above horizontal. All rotator drums are constructed of enamel-coated stainless steel for corrosion resistance. Prices for the bottle and tube rotators range from £480–1,200 (UK).



Day-Impex tissue culture rotators provide variable speed and angle of rotation.

Save space in your fridge/freezer with the new plastic **square media bottles** from Nalge Co., which are now available in 30- and 60-ml sizes (*Reader Service No. 103*). The PETG square bottles do not break or shatter like glass and have O_2/CO_2 barrier properties that prevent outgassing and destructive pH shifts in the stored media, says Nalge. The transparent bottle and leakproof linerless closure are made of materials that are non-toxic and non-pyrogenic. Aseptic technique is facilitated by the deep closure and narrow-mouth design, and the pouring ring on the neck aids dispensing. Researchers can choose from three different resins for bottles that are autoclavable, chemical and temperature resistant. The 30-ml and 60-ml bottles are $81.60 and $105.60 (US), respectively, for a case of 96 bottles.

Space-saving square media bottles from Nalge.

In a spin

Measuring only 12 cm in diameter, the MC 200 NanoFuge **microcentrifuge** is, says Hoefer, ideal for applications requiring spinning at low *g*-forces (*Reader Service No. 104*). The $355 (US) NanoFuge can hold up to six 1.5- or 2-ml microcentrifuge tubes or, when fitted with an adaptor, will hold tubes as small as 0.25, 0.4 and

The MC 200: Hoefer's tiny microcentrifuge for spinning at low *g*-forces.

0.5 ml. Stated applications include spinning down droplets from caps and walls of microcentrifuge tubes, blood, urine and tissue centrifugation, and the sedimentation of bacterial cells. Closing the lid of the instrument initiates the spinning process and opening it stops it. The NanoFuge can operate at a maximum speed of 5,000 r.p.m. with a *g*-force of 200.

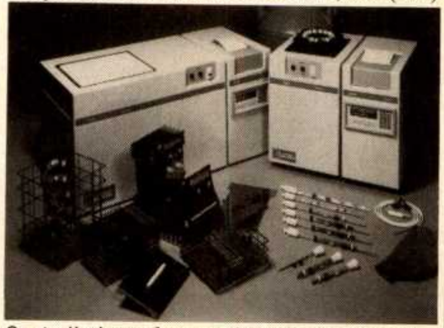
Scientific Equipment Design has launched a new generation of **refrigerated centrifuges** that employ solid-state refrigeration technology, eliminating the need for CFCs, bulky compressors or gas refrigeration (*Reader Service No. 105*). The £2,985 (UK) 20R high-speed microcentrifuge can be supplied with a large number of rotor options that will hold anything from 120 microtubes to six 25-ml tubes. The 20R can operate at maximum speeds of 11,000 r.p.m. The £3,796.50 (UK) 30R refrigerated benchtop centrifuge has a capacity of two litres and can operate at speeds of 3,000 r.p.m. or 5,000 r.p.m. if fitted with smaller rotors. Cooling is provided down to 2 °C and the temperature can be set to within 0.1 °C by the digital temperature display/control. According to SED, the bowl temperature should equilibriate within 10 minutes of switch-on. Safety features of the 20R and 30R include an automatic out-of-balance indicator and safety cut-out, dual-lid interlock and three-position brake.

Frozen assets

Transgenic Sciences, Inc. has developed Cryozyte — a **cryoprotectant system** designed for the long-term, low-temperature storage of mouse embryos (*Reader Service No. 106*). The procedure takes about an hour and permits the ultra-rapid cooling of mouse embryos without ice crystallization. Once preserved in Cryozyte, TSI says the embryos can be stored indefinitely in any standard liquid nitrogen containers that accommodate canes. Viable embryos are incubated in equilibrium medium and then loaded into plastic straws that are prefilled with vitrification medium. Embryos are recovered by thawing at room temperature, followed by incubation in dilution and rehydration media. The $235 (US) kit comes complete with straws, plugs, step-by-step protocol guide, and all the necessary solutions for the cryopreservation of 375 mouse embryos.

Cryopreserve mouse embryos with Transgenic Sciences' Cryozyte kit.

Two new **controlled-rate freezer units** — Kryo 10/16 and 10/1.7 — are available from Planar Biomed (*Reader Service No. 107*). The freezers consist of a benchtop controller with a separate and insulated freeze-chamber module which is filled by the programmed transfer of liquid nitrogen. The Kryo 10/16, costing $11,500 (US) for the basic unit, has a 16-litre capacity and is suitable for freezing blood bags, ampoules and straws. The $11,750 (US) Kryo 10/1.7 model has a smaller capacity but straws and ampoules can be placed both horizontally and vertically. The temperature range in both units is +40 °C to −180 °C with cooling rates variable between 0.01 °C per minute to −50 °C per minute. The microprocessor-based programmer monitors the chamber and sample temperature with platinum-resistance thermometers: any malfunctioning is indicated on the display. Up to ten sets of program parameters can be stored.



Controlled-rate freezers from Planar Biomed.

Jencons Scientific Ltd are the sole distributors in the UK for the Taylor-Wharton **cryostorage systems** — the K Series — where K denotes the storage capacity in thousands of 2-ml vials (*Reader Service No. 108*). The K Series consists of 3K, 8K, 17K and 27K units, although a 5K unit will be available in early 1990. The refrigerator units can be provided with dedicated inventory systems for both liquid- and vapour-phase sample storage. Automatic control options — alarm only, autofill or full automatic control — for monitoring the level of refrigerant are available for the 8K, 17K and 27K models. The modular design of the K Series allows several units to be connected up to a single liquid nitrogen source, while each can remain under independent control.

DNA extraction

Using **glass adhesion methods**, extract DNA from agarose, separate out unincorporated nucleotides, remove organic solvents, isolate DNA from minipreps and concentrate DNA with the USBioclean kit from USB (*Reader Service No. 109*). Based on the methods of Vogelstein and Gillespie, 2 μl of the glass powder v typically bind 1 μg of DNA with 80 p cent recovery of the starting material, sa USB. The $80.50 (US) USBioclean comprises glass powder suspension, N solution, concentrated rinse buffer, Tr Acetate-EDTA electrophoresis buff and pipette tips. It is unsuitable for DN extractions from acrylamide gels, but c be used for both low- and high-m agarose. DNA that has been extracted this method, which, USB says, tak about 20–25 minutes, can be used f restriction digests and other enzyma manipulation.

Perkin-Elmer Cetus is offering th Isogene kit for the **extraction and purific tion of DNA** (*Reader Service No. 110*). Th method uses a DNA binder, which, th company says, has a higher capacity tha the glass-milk variety — typically 5 μg DNA is bound using 10 μl of binde Recoveries of bound DNA of 80–95 p cent can be achieved after two elution with 20 μl of distilled water. Stated appl cations for Isogene include the extractio and purification of DNA from agaro gels, extraction of plasmids from lysate desalting and/or concentrating DN solution, separating DNA from enzym inhibiting impurities and the replacemen of conventional alcohol precipitation c column-based methods for DNA purifica tion. Isogene kits are supplied with DN binder, NaI solution, washer buffer con centrate — sufficient for 100 assays, say Perkin-Elmer.

Detection systems

Tropix, Inc. has introduced two new optical **luminometers** for use with an chemiluminescent or bioluminescen reaction (*Reader Service No. 111*). Th $4,500 (US) ILA 911 single-well counter i manually operated while the $12,500 (US ILA 912 is a fully automated system tha can process up to 220 tubes. Both instru ments are fitted with a 1.125-inch photon counting photomultiplier, which, Tropi says, has a 20,000,000 count per secon rate maximum and has a programmabl optical sensitivity selection. The spectra response range is 320–500 nm. Afte insertion of the tube in the countin chamber, background measurements optional injections, shutter apertur selection and integration are automatically sequenced. The built-in microcomputer includes immunoassay, kineti and DNA probe data reduction protocols and can store up to 30 user-definable protocols. Each luminometer is equipped with a thermal graphics plotter. External computers and data reduction systems can be linked up to the luminometer via the RS232 port.

For the simultaneous **separation of ionic, neutral or polar analytes**, Dionex Ltd recommends the CES 1 Capillary Electrophoresis System (*Reader Service No. 112*). The CES 1 features a fully programmable dual-polarity power supply with the choice of three control modes — volt, current or power — as well as multistep gradients in all three modes. Also featured are three automated injection modes: electromigration, gravity and pneumatic. A random-access 40-position autosampler provides automatic rinsing of vials and capillaries and buffer changing. Separation takes place in an open capillary using electrophoretic migration and electro-osmotic flow to separate and elute components past an on-column detector. The CES 1 can monitor absorbance at wavelengths between 190 and 800 nm, and a bandwidth of 6 nm ensures detection of samples with injection volumes in the order of 1–10 nanolitres, says Dionex. Programme parameters input via the instrument's keypad can be viewed on the video display and methods can be stored in the non-volatile memory. Hard copy is output in a chromatography-like format.

Capillary electrophoresis from Dionex.

The collaborative efforts of Intracel Ltd, Nikon and Newcastle Photometric Systems have produced the Epi Fluorescence Systems for **single and dual wavelength fluorochromes** (*Reader Service No. 113*). The Epi Fluorescent System is based on the Diaphot inverted microscope, a series of bolt-on accessories that include photon counters, excitation and emission filter changers, and control software that allows the detection and quantification of Ca^{2+}, H^+, Mg^{2+}, Cl^- and Na^+ within single cells. For dyes with dual emission characteristics, a beam splitter is used to separate the two wavelengths, and two photomultipliers are used to detect the wavelengths simultaneously. For dyes that require excitation at two wavelengths, filter changers are used in conjunction with a single photomultiplier system. The software provides control of the arc and wheel filter changers and calibration of the photomultipliers. Experimental data can be shown on the computer screen and stored on disc.

Catalogues and services

Over 100 new products are listed in the free 1990 Sigma Cell Culture **catalogue** (*Reader Service No. 114*). The 248-page catalogue lists Sigma's complete line of tissue culture products, including 35 new products for plant tissue cultures, liquid and powdered media, Hybri-max products, sera and sera replacements, cell culture-tested reagents, biochemicals and equipment. In addition to the product list, the catalogue includes an expanded section of technical information, including commonly used techniques, stability studies, a guide to antibiotic use, and instructions for supplementing liquid media.

Sigma's 1990 cell culture catalogue.

Trouble shoot procedures for transfer methods when using hybridization membranes from Micron Separations, Inc. with their latest **protocol guide** (*Reader Service No. 115*). The guide outlines procedures for Southern, Northern and Western blotting, colony hybridizations, plaque lifts, nucleic acid dot/slot blotting, alkaline blotting, and the use of biotinylated probes with MSI nylon and supported nitrocellulose membranes. The guide features two new sections: how to select an appropriate membrane to suit the transfer procedure and ways to reduce background during the transfer process.

Neuropharm Ltd are offering a **neurotoxicity evaluation service** using the leech brain as a model system (*Reader Service No. 116*). Toxicological screening of compounds has, until now, been based almost exclusively on mammalian studies. Neuropharm are using the simpler nervous system of the leech to carry out acute neurotoxicity tests, which include acute dose response, type of response, mechanism of ion channel and receptor response. Neuropharm, emphasizing the advantages of using the leech, point out that leech neurons are large, easily accessible and individually identifiable. They can be removed as a whole or in part, isolated for several hours and can be impaled with more than one microelectrode.

Neurotoxicity testing service using the leech brain, from Neuropharm.

Custom Immunology is BAbCO's new brochure that describes their **contract antibody developoment service** (*Reader Service No. 117*). The brochure outlines custom services and protocols provided by BAbCO for polyclonal antibody generation in small and large animals, hybridoma cell-line development, ascites fluid scale-up production of mAbs under GMP guidelines, GLP animal model and veterinary science studies. Other services include antigen preparation, protein isolation and purification, peptide-carrier conjugation, antibody fragmentation, biotinylation, enzyme and fluorescent labelling. ☐

These notes have been compiled by Diane Gershon, from information provided by the manufacturers. To obtain additional information about these products, use the reader service card bound inside the journal. Prices quoted are sometimes nominal, and apply only within the country indicated.

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Reader Service No.15

Can student numbers be doubled?

Richard Pearson

The reduced numbers of students in higher education likely to result from demographic changes in the 1990s wil have to be countered by changes in attainment and participation rates.

LAST month, the Employment column showed how the output of the UK higher education system has trebled over the past three decades and how this expansion continued even through the previous demographic downturn which started in the late 1960s.

The question for the 1990s is whether the present output of 120,000 graduates a year can be doubled over the next 25 years as suggested by the Secretary of State for Education.

As a starting point, it is important to recognize that while the decade is ending with shortages of graduates, particularly in technological subjects, a significant number of graduates are unemployed and about one in four say they are underemployed.

The demand for good graduates is expected to increase, although it is not possible to forecast precise numbers or the balance between subjects. If demand continues to grow as it has over the past decade, demand could be 30 per cent higher at the end of the century than it is today.

The number of new graduates is expected to increase by more than 5 per cent by 1992. These students are already on their courses. Thereafter the numbers will depend on staying-on rates in education, examination achievements and the attractions of higher education to the young, particularly women, and to vocationally qualified and mature entrants.

Changes

Without changes in these factors, demographic change alone within the social classes will reduce the number of entrants to higher education by 10 per cent over the period 1990–2000.

- If, however, educational attainment levels rise by a single percentage point, a rate of improvement faster than that achieved in the 1980s, then the number of entrants would be four percentage points higher.
- If the A-level attainment rate throughout the United Kingdom were to equal that in Scotland, the numbers would be 31 percentage points higher.
- If the attainment rates of social classes III to V rise to match those of social classes I and II, the numbers would be 65 percentage points higher.
- If women's participation matches that of men, the numbers should be seven percentage points higher.
- If the participation rate of the vocationally qualified rises to match that of A-level students, the numbers would be 11 percentage points higher.
- Finally, if the participation rate of mature students grows by 50 per cent, the numbers would be 15 percentage points higher in the year 2000.

Scenarios

These changes were combined into two scenarios to illustrate what might be achieved in the next decade[1]. The first — rising participation — assumes that attainment rates will rise faster than in the 1980s, participation rates will rise, the number of vocationally qualified entrants will double, the participation rates of mature students will rise by 50 per cent and student loans will not suppress student demand for higher education. Under this scenario, the number of entrants would be 33 percentage points higher in the year 2000.

The alternative scenario, 'constrained expansion', assumes that improvements in attainment rates will continue, but at a rate nearer than of the 1990s, employer competition for school leavers will reduce participation rates, the number of vocationally qualified will be stable and the mature participation rate will rise by 25 per cent. Under this scenario, all the main factors still show improvements on the 1980s, but the effect would be only just enough to counteract the demographic downturn, with student numbers in 2000 being the same as in 1990.

It can be seen that significant changes in attainment and participation rates will be required simply to counteract the demographic effect over the next decade.

To meet the Secretary of State's wish to double student numbers would require changes along the following lines: significant increases in staying-on rates in education at the age of 16 to ensure that A-level attainment rates rise from under 14 per cent to more than 20 per cent, which is almost the level in Scotland; A-level attainment rates of social classes II–V rising by a further half; female participation matching that of males; participation by the vocationally qualified rising significantly to reach the A-level rate; and the mature participation rate increasing by more than 50 per cent.

These represent big improvements on the rates of change seen in the past decad and would result in fewer young peopl being available for employment in th labour market.

Competition

Student demand for higher educatior may, however, be reduced by the growin competition between employers an higher education institutions for th reduced number of school-leavers. Man employers are improving schools liaison and training and career prospects, and are raising salaries in order to maintain school-leaver intakes. They are also widening their recruitment nets to include more women and re-entrants to the labour market. Thus there will be increasing competition to retain pupils in the education system, and to attract both traditional and nontraditional entrants into higher education. The introduction of student loans and a reduction in the size of the maintenance grant is also likely to reduce the short-term attraction of higher education.

The issue for policymakers in higher education, industry and government is how to stimulate student demand and how to organize higher education and provide it with resources to cope with much larger numbers.

Even if student numbers increase, it is likely, assuming that the economy continues to grow, that the demand for good graduates will exceed the supply and that shortages will continue, at least in the first half of the 1990s. Much of this demand will focus on the traditional young graduate with a reasonable class of degree and broad basic skills. Any increase in the supply will include an increasing proportion of nontraditional graduates who, while they may not be 'worse', will be 'different'. A challenge for students, higher education and employers will be to ensure that the attributes of the new supply and the nature of employer demand coincide, otherwise there will be a significant number of graduates who find it difficult to enter what they regard as suitable employment in the 1990s. ☐

Richard Pearson is at the Institute of Manpower Studies, Mantell Building, University of Sussex, Falmer, Brighton, Sussex BN1 9RF, UK.

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[1] Pearson, R., Pike, G., Gordon, A. & Weyman, C. *How Many Graduates in the 21st Century? — The Choice is Yours* (IMS, Brighton, 1989).

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Curriculum vitae and names of references should be submitted to:

Dr. Bruce Stillman
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(NW4606)A

RTMENT OF BIOCHEMISTRY
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s Molecular Biology Group directed by Drs S. and A. Kingsman
a cell biologist to join a multi-disciplinary programme aimed
rstanding and inhibiting the functions of HIV structural and
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ts trained in other areas are encouraged to apply.

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vill be at an appropriate point on the post-doctoral RA1A scale
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resses of two referees, should be sent, as soon as possible to:

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ROYAL PERTH HOSPITAL

An Equal Opportunity Employer

Senior Research Officer

Applications are invited for an NH & MRC funded position of Senior Research Officer within the Department of Clinical Immunology at Royal Perth Hospital. The appointment will be for up to five years with opportunity for promotion according to NH & MRC criteria. The project relates to regulatory factors relevant to autoimmune disease and immunodeficiency. A PhD or MD with experience in cellular immunology is required to complement others with experience in cloning and mapping of MHC genes. The appointee should be able to supervise a laboratory with several staff and students and will need to be capable of attracting competitive funding. The salary will range from $40,463 to $52,170 depending on qualifications.

APPLICATIONS BY THE 5TH JANUARY 1990 INCLUDING CURRICULUM VITAE, REPRINTS AND THREE PROFESSIONAL REFEREES SHOULD BE ADDRESSED TO PROFESSOR RL DAWKINS, DEPARTMENT OF CLINICAL IMMUNOLOGY, ROYAL PERTH HOSPITAL, BOX X2213, GPO, PERTH WA 6001, WESTERN AUSTRALIA.

(2263)A

BioResearch Ireland is a contract research organization formed to commercialise biotechnology research in Irish universities and research institutes and now invites applications for:

RESEARCH SCIENTIST
VIROLOGY

A position is available with the research group studying the "Pancreas Disease Syndrome" of Atlantic Salmon at the National Diagnostics Centre based in University College Galway.

Applicants should have a post-graduate degree and/or several years experience in virology and cell culture. Expertise in electron microscopy would be an advantage.

Please apply before *21st December 1989* by sending c.v. and names of three references to:

Mary Gillick,
BioResearch Ireland
EOLAS
Glasnevin
Dublin 9
Telephone 01-370 177

BioResearch Ireland operates as a division of EOLAS – the Irish Science and Technology Agency.

BioResearch Ireland

(2285)A

dkfz

The Foundation Deutsches Krebsforschungszentrum Heidelberg (German Cancer Research Center) is a National Research Center (Großforschungseinrichtung), mainly funded by the Federal Ministry of Research and Technology and by the state of Baden-Württemberg. About 1400 people work in the center including more than 250 scientists. At present the following postdoctoral positions are available for scientists trained in natural sciences or medicine in the following.

Papillomaviruses in malignant tumors

Dr. E.-M. de Villiers (Applied Tumorvirology)

The program entails research on the role of papilloma viruses in malignant tumors under special emphasis on pathogenetic aspects. Experience in molecular biological methods is desired.

Growth control of normal and malignant Lymphocytes

Prof. Dr. P.H. Krammer (Immunogenetics)

Research on the role of cell surface molecules in growth control of normal and malignant lymphocytes. Experience in cellular immunology, immunochemistry and/or molecular biology is desired.

Applicants should have a Ph.D or M.D. degree and must be younger than 32 years. Experience in the relevant fields is a prerequisite for a successful application. The contracts are limited to a maximum of 3 years with a net salary of about 56.000,-- DM per year.

Written applications with curriculum vitae copies of diplomas, list of publications and further information about research interests should be sent to our Personalabteilung within three weeks after publication.

Im Neuenheimer Feld 280
D – 6900 Heidelberg
Telefon 06221/4841

(W6796)A

MEDICAL RESEARCH COUNCIL

National Institute for Medical Research
Physiological and Neural Mechanisms Group

BIOSTATISTICIAN/STOCHASTIC MODELLER

Applications are invited for a short-term (three-year) post-doctoral appointment to the scientific staff of the Laboratory of Mathematical Biology. The research, which will be under the supervision of Dr. T.B.L. Kirkwood (Head of Laboratory), will involve the design and statistical analysis of experiments in collaboration with other groups in the Institute. Candidates with experience of stochastic methods are particularly encouraged to apply as there are major opportunities for research using stochastic models in our work on DNA and protein sequences and on cell and virus population dynamics. Experience of biomedical research would be an advantage but is not essential as relevant training will be given.

The position is available immediately. Informal enquiries may be made by telephoning Dr. Kirkwood on 01-959 3666 Ext. 2294. The salary will be in the range £12,381 to £20,469 (starting salary dependent on experience) plus £1,650 per annum London Allowance. MRC Pension Scheme optional.

Applications including a curriculum vitae, list of publications and the names and addresses of two professional referees should be sent to **Mr. C.R. Russell, Administrative Manager, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA,** not later than 29th December, 1989, quoting reference number PMB/0711.

The MRC is an Equal Opportunities Employer. (2261)A

NATIONAL INSTITUTES OF HEALTH

POSITIONS FOR STAFF FELLOWS (TENURE TRACK) AND POST-DOCTORAL FELLOWS

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III. **AUTOIMMUNE DISEASES** - Cloning, expression and characterization of autoantigens involved in insulin-dependent diabetes mellitus, lupus erythematosus, rheumatoid arthritis, and Graves' disease.

Positions available immediately. Experience in molecular biology desirable. Growing program with opportunity for Staff Fellows to have technical and/or post-doctoral support. Salary commensurate with experience (range $25,000 to $50,000 per year). Send Curriculum Vitae and names of references to Dr. Lois Salzman, Deputy Director, Intramural Research Program, National Institute of Dental Research, National Institutes of Health, 9000 Rockville Pike, Building 30, Room 121, Bethesda, MD 20892.

NIH IS AN EQUAL OPPORTUNITY EMPLOYER (NW4670)A

UNIVERSITY OF MANCHESTER
CENTRE FOR EPITHELIAL MEMBRANE RESEARCH AND DEPARTMENT OF MEDICINE (GASTROENTEROLOGY)
HOPE HOSPITAL

Applications are invited for these posts on a three year project, funded by the Sir Jules Thorn Charitable Trust.

1) **POSTDOCTORAL RESEARCH ASSOCIATE**: to investigate interactions between second messenger pathways in cultured human colonocytes and their role in regulating epithelial secretion. Experience in one or more of the following areas would be an advantage: epithelial transport mechanisms, cellular signalling, the application of single cell fluorescence techniques. Salary on the Research IA scale (£10,458-£16,665 p.a.) according to qualifications and experience.

2) **RESEARCH TECHNICIAN**: to join a group investigating the intracellular regulation of intestinal secretion using cultured epithelial cell lines. Practical experience in mammalian cell culture is desirable though not essential. Salary on the University Technician 4/5 scale (£8,645-£10,632 p.a.) according to qualifications and experience.

Applications, including a full curriculum vitae and the names and addresses of two referees, should be sent to **Dr G. Warhurst, Department of Medicine, Clinical Sciences Building, Hope Hospital, Salford M6 8HD** (061 789 7373, Ext 4403) from whom further information can be obtained. Closing date January 5th, 1990. The University is an equal opportunites employer. (2250)A

ROYAL POSTGRADUATE MEDICAL SCHOOL
(University of London)

BIOCHEMICAL PHARMACOLOGIST

SENIOR RESEARCH OFFICER

Applications are invited from postdoctoral scientists, for the post of senior research officer. The research is funded by a Programme Grant from the Wellcome Trust and relates to the function of G-proteins in signal transduction. A background in biochemistry, pharmacology or a related subject is essential, and experience in the field would be an advantage. The position is available to applicants from all EEC countries, and the salary will be in the range of £12,381 – £14,703 (plus £1,650 London Allowance). The post is available to end of October 1992 in the first instance. Informal enquiries should be made to Professor J MacDermot, Department of Clinical Pharmacology (01-740 3219).

Application forms and further details are available on request from the **Personnel Officer, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0NN. Please quote ref: AEU8. (tel: 01-740 3204).**

(2267)A

JAMES COOK UNIVERSITY OF NORTH QUEENSLAND
Townsville, Australia

MECHANICAL ENGINEERING

In order to meet the demands of continuing technological development in the North Queensland region, James Cook University is expanding its two-year feeder course in Mechanical Engineering to a full four-year degree program. The third year of the course was offered for the first time in 1989 and the fourth year will be offered in 1990.

The undergraduate program that is being developed is innovative in a number of ways. The two senior years of the course, for example, are largely project-oriented and have a strong emphasis on engineering design, contemporary technology, and the development of entrepreneurial and management skills.

Research and consulting activities will focus on the special problems and unique opportunities presented to industry in the tropics and in North Queensland in particular. Existing industries in the region such as agriculture, mining and manufacturing are being supplemented with high-technology ventures in fields such as marine technology, energy technology, tourism and recreation and aerospace engineering.

Applications are invited for the

FOUNDATION CHAIR IN MECHANICAL ENGINEERING

Applicants must have appropriate academic qualifications and a demonstrated ability to implement an innovative program in mechanical engineering education, together with an international reputation for research and scholarship.

The appointee will be expected to provide academic leadership and to liaise closely with local industry in generating co-operative research and teaching programs.

A Division of Mechanical Engineering is currently located in the Department of Civil and Systems Engineering, but a separate Department of Mechanical Engineering will be established not later than 1 July 1990. The appointee will be expected to take up duty as soon as possible after appointment.

The salary for Professor is A$65,837 a year.

Consultative and other outside paid work is encouraged, and the University's policy on the matter is generous.

Reference No. 89205.

Further particulars and application forms are available from **Appointments (37251), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF**, or from the **Registrar, James Cook University of North Queensland, Townsville, Qld. 4811, Australia,** with whom applications close on *31 January 1990*. (W6785)A

The Norwegian Research Council for Science and Humanities (NAVF) invites application from

SENIOR SCIENTIST IN VIROLOGY

for the position of research leader at the National Centre for Research in Virology in Bergen, Norway. The Centre of Virological Research is associated with the Virus Laboratory in the Department of Microbiology and Immunology and with the Center of Biotechnology of the School of Medicine, University of Bergen. The research leader will be responsible for implementation of an education and research development programme outlined by the Council for Medical Research under NAVF. He/she will be affiliated with the University of Bergen through a professorship position and will have excellent facilities to conduct own research, both independent and collaborative, and to build up her/his own research group.

The centre will have excellent laboratory facilities, housed together with the Virus Laboratory and the Center of Biotechnology in a new high technology centre to be opened in late 1990. Present research activities in virology are primarily concentrated on molecular biological and immunological aspects of retrovirus/HIV and herpes simplex virus infections in collaboration with research groups in UK and USA. Four senior and 6-8 junior scientists are connected to these projects in addition to those currently working at the centre.

It will be required that the research leader has broad scientific experience in virology with emphasis on molecular virological research relevant to human medicine. Significant experience in teaching and in research supervision at pre- and postdoctoral levels is mandatory.

The salary will be determined according to qualifications and experience.

For further information concerning the position write to **Professor Gunnar Haukenes, National Centre for Research in Virology, Armauer Hansen Building, N–5021 Bergen, Norway.**

Applicants should send their curriculum vitae and list of publications together with a description of their plans for the research activity, to Professor G. Haukenes, address as above, by January 15, 1990.

(W6788)A

CELL BIOLOGY
DEVELOPMENTAL NEUROBIOLOGY

Two senior level faculty positions
Department of Biology
University of California, Riverside

The Department of Biology, University of California, Riverside, invites applications for two openings at the senior level. One is in cell biology, at the Associate or Full Professor level; the other is in developmental neurobiology at the Full Professor level. The appointments will be effective July 1, 1990. Applicants should have an active research program, and an established reputation with a proven record of competing successfully for extramural funding. Demonstrated interest and ability in teaching is sought. Salary will be competitive and will depend on qualifications and experience. Modern research space and access to major equipment such as TEM, SEM, and staffed biotechnology and analytical chemistry instrumentation facilities are available.

CELL BIOLOGY

We are interested in all areas of research in cell biology. Preference will be given to scientists applying modern molecular approaches to the study of the cell and with interests in: cell/cell interactions, protein sorting and assembly, cytoskeletal structure and function, or extra-cellular matrix. Applications should be received by January 15, 1990. Please send *curriculum vitae*, summary of research and teaching interests, and arrange to have 3–5 referees send letters to: **Cell and Molecular Biology Search Committee, Department of Biology, University of California, Riverside, CA 92521.**

DEVELOPMENTAL NEUROBIOLOGY

Acceptable areas of resaerch include, but are not limited to, gene regulation and expression during neural development, neural differentiation and the development of integrative mechanisms, synaptogenesis, regeneration, mechanisms of learning and memory, and analysis of neural connectivity in response to environmental conditions, hormones or other factors. The successful candidate will be expected to assume a leadership role in the interdepartmental Neurobiology Program. Applications should be received by January 15, 1990. Please send *curriculum vitae*, summary of research and teaching interests, and arrange to have 3–5 referees send letters to: **Develpmental Neurobiology Search Committee, Department of Biology, University of California, Riverside, CA 92521.**

The University of California is an Equal Opportunity/Affirmative Action Employer and minority and female candidates are encouraged to apply. (NW4673)A

University of Utrecht

FACULTY OF VETERINARY MEDICINE

The Faculty of Veterinary Medicine of the University of Utrecht, The Netherlands, invites applicants for the position of

vac.nr. 70035

Full Professor in Parasitology

Applicants are expected to meet the following requirements:
- general and specific knowledge of parasitic infections and diseases in domestic animals, including parasitic zoonoses;
- expertise in molecular-biological, biochemical and/or immunological aspects of host-parsite relationships;
- experience in research management;
- experience in teaching;
- willingness to collaborate with other disciplines;
- willingness to serve on faculty boards and committees.

Candidates should:
- be a graduated veterinarian, medical doctor or (molecular)biologist;
- possess a Ph.D. or equivalent academic degree;
- be specialized in parasitology as evidenced by publications of high standard;
- be willing to learn Dutch within a reasonable period of time.

Further information is available on request.

The salary will be determined according to the experience of the applicant.

Application, accompanied by a full curriculum vitae and a list of publications, or suggestions of suitable candidates, should be confidentially sent to:

Prof. dr. Simon G. van den Bergh, dean, Faculty of Veterinary Medicine, P.O. Box 80.163, 3508 TD Utrecht, the Netherlands.

With equally qualified applicants preference will be given to woman.

(W6789)A

CELL AND MOLECULAR BIOLOGY POSITION:

The Department of Biological Sciences and the Center for Gene Structure and Function is recruiting for two positions for Cell or Molecular Biologists as tenure track faculty members. Appointment at any professional rank is possible depending on the qualification of the successful applicant. Successful candidates will be expected to establish an independent research program and to participate in team-taught courses in their area of research at the undergraduate and/or graduate level. Suitable start-up and relocation funds are available. Send curriculum vitae, a statement of research interests and names of references to: **Dr. Joseph S. Krakow, Chairman, Search Committee, Department of Biological Sciences, Hunter College, City University of New York, 695 Park Avenue, New York, NY 10021.** Hunter College is committed to participation in science at all levels by minorities and women.

(NW4693)A

CONDENSED MATTER EXPERIMENTAL PHYSICISTS

COLUMBIA UNIVERSITY DEPARTMENT OF PHYSICS

The Department of Physics of Columbia University is searching for outstanding condensed matter experimental physicists at the assistant professor level.

The successful candidates will be provided with startup funds to suit the requirements of their research programs. There is no restriction on the subfield that will be considered. Tenure positions will be available for some of the physicists in this program who develop truly outstanding research programs and who also demonstrate excellent teaching skills. We will also consider applications, at this time, from more senior experimental condensed matter physicists who presently have the qualifications for a tenure position.

Finally we will consider applications from small, established, highly successful groups of experimental physicists who might be interested in moving as a group to Columbia. These positions will remain open until they are filled. Persons or groups interested in being considered for these positions should send their curriculum vitae, publication list, and a statement of their research interests to:

Professor S. Nagamiyn
Condensed Matter Search Committee Chairman
Department of Physics
Columbia University
Box 3 Pupin
New York, N.Y. 10027.

They should also arrange to have, at least three letters of recommendation sent to the same address.

Columbia University is an equal opportunity-affirmative action employer. Women and members of minority groups are encouraged to apply for these positions. (NW4624)A

UNIVERSITY OF GLASGOW

CHAIR OF ARCHAEOLOGY

Applications are invited for the Chair of Archaeology in the University of Glasgow which fall vacant on 1st October, 1990 on the retiral of Professor Leslie Alcock.

The principal research and teaching interests of the Department of Archaeology lie in North-West Europe, without chronological limits, but with a special interest in Britain and Ireland. There is a strong commitment to archaeological science; and both the practical and theoretical aspects of archaeology feature strongly in the Department's teaching and research.

Further particulars may be obtained from the **Academic Personnel Office, University of Glasgow, Glasgow G12 8QQ,** where applications (3 copies; 1 copy in the case of overseas applicants), giving the names and addresses of three referees, should be lodged on or before 15th January, 1990.

In reply please quote Ref. No. 6774. (2271)A

RESEARCH FELLOW

(Anthelmintic Pharmacodynamics)

A$31,525-A$38,567

The CSIRO Division of Animal Health, Glebe, NSW Australia is seeking a research fellow for a term of three years to undertake research to characterise the relationship between molecular structure of antiparasitic agents (eg. benzimidazoles) and effects on absorption, gastric secretion and recycling of these compounds to the gastrointestinal tract of sheep.

Applicants should be self motivated and possess a Ph.D degree or equivalent qualifications in physiology and pharmacology.

Applications quoting reference No. KGL14 and the name/address of two professional referees, to the

McMaster Laboratory,
Private Bag No. 1,
P.O. Glebe, NSW Australia 2037
Fax: (02) 692 8561
by 16 February 1990.

CSIRO
AUSTRALIA

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER (W6794)A

EAST BIRMINGHAM
HEALTH AUTHORITY

EAST BIRMINGHAM
HOSPITAL – REGIONAL
CYTOGENETICS
LABORATORY

PRINCIPAL CLINICAL CYTOGENETICIST

pplications are invited for the st of Principal Clinical Cytoge- ticist in the Regional Cytogene- cs Laboratory based at East irmingham Hospital. This la- oratory provides a comprehen- ve cytogenetic service for a popu- tion of around 2.6 million. The ain duties of the Principal Cyto- eneticist will be related to the evelopment and running of the ytogenetic services, but facilities r research exist by collaboration ith Cytogenetic and Molecular enetic research groups within e Department.

Informal enquiries are welcome nd application forms may be ob- ined by contacting Professor Maj ulten, Regional Genetic Ser- ices, East Birmingham Hospital, ordesley Green East, Birming- am B9 5ST. Tel. 021 766 6611 xt. 2450.

Closing Date: 29th December 989. (2279)A

Ciba-Geigy is a major pharmaceutical company with an active interest and commitment to molecular biology. The Molecular Biology Department forms part of the Biotechnology Section located in the Company's main research facility in Basle, Switzerland. Applications are invited for a position as

Molecular/Cell Biologist

as research scientist in the Molecular Biology Department. The successful candidate will join a well-established unit and assume responsibility for a laboratory involved in developing and applying molecular/cell biology techniques for the identification and expression of new proteins of potential therapeutic/pharmaceutical interest.

We are seeking to recruit an independent, innovative research scientist with a Ph.D. and at least 2-3 years postdoctoral experience, who is motivated to work effectively in a highly-interactive, multidisciplinary research environment. Preference will be given to candidates with expertise in molecular biology who have had additional training in mammalian cell biology and/or immunology.

Applications, including a c.v., a summary of relevant research background and the names of two referees should be sent under ref. "Nature 2871" to **H. Schmid, CIBA-GEIGY Ltd., Personnel Department, P.O. Box, CH-4002 Basle.** (W6790)A

CIBA–GEIGY

Postdoctoral Training — Insect Molecular Biology

Two postdoctoral positions are available for studies on the development of recombinant baculoviruses for biological control of lepidopteran pests and the establishment of methods for silkworm embryo transformation. Candidates should not have more than two years of previous postdoctoral training and should have experience with recombinant DNA methodology. Annual starting stipends $22,000-24,000, depending on qualifications. Applications containing curriculum vitae, a brief summary of previous training experience and research goals, as well as names and addresses of three referees should be sent to:

Dr. K. Iatrou, Department of Medical Biochemistry, Faculty of Medicine, The University of Calgary, 3330 Hospital Drive N.W., Calgary, Alberta T2N 4NI, Canada.
(NW4687)A

PLANT ECOLOGIST

Tenure-track Assistant/Associate Professor position starting 1990-91 academic year. Candidate must have a strong field orientation and to bring modern approaches and techniques to research on terrestrial problems in Hawaii. The Botany Department's commitments include the investigation of mechanisms in the ecology and evolution of Hawaiian ecosystems and species. Responsibilities include research, teaching and service; courses could include introductory botany or biology, upper-division ecology and a specialized graduate course. Send curriculum vitae, reprints, statement of research and teaching interests and names of three referees to: **Chair, Ecology Search Committee, Botany Department, University of Hawaii at Manoa, 3190 Maile Way, Honolulu, HI 96822.** Closing date for receipt of applications: February 15, 1990. Inquiries accepted by FAX: 808-943-0380. An Equal Opportunity/Affirmative Action Employer. (NW4705)A

THE UNIVERSITY OF ADELAIDE
South Australia

invites applications from both women and men for the following position:

RESEARCH ASSOCIATE/RESEARCH FELLOW IN BIOCHEMISTRY

(Ref: 6925) in the DEPARTMENT OF BIOCHEMISTRY, to work on the molecular analysis of barley yellow dwarf virus in relation to the Yd2 resistance gene of barley. The general approach is to characterize strains of the virus which can and cannot overcome the natural resistance provided by this gene. Data obtained are required for the rational development of protecttion measures against this important virus of cereals.

Applicants should have, or be about to complete a Ph.D. and should have significant experience in molecular biology, preferably in the manipulation of nucleic acids. Experience in plant viruses is desirable but not essential.

The position is funded by the Australian Research Council and is available from 1 January 1990, for up to three years.

Further information concerning the duties of the position may be obtained from Professor R.H. Symons, Department of Biochemistry, telephone (08) 228 5354, Fax. (61 8) 224 0464.

INFORMATION about the general conditions of appointment and selection criteria may be obtained from the Senior Assistant Registrar (Personnel) at the University or from Appointments (37220), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF.

SALARY per annum: Research Associate A$27,953 × 5 — A$31,808; Research Fellow A$32,197 × 7 — A$41,841.

APPLICATIONS, IN DUPLICATE quoting reference number 6925 and giving full personal particulars (including whether candidates hold Australian permanent residency status), details of academic qualifications and names and addresses of three referees should reach the Senior Assistant Registrar (Personnel) at the University of Adelaide, GPO Box 498, Adelaide, South Australia, 5001, (Telex UNIVAD AA 89141, Facsimile 61 8 224 0464) not later than **8 January 1990**.

The University reserves the right to make enquiries of any person regarding any candidate's suitability for appointment, not to make an appointment or to appoint by invitation.

THE UNIVERSITY OF ADELAIDE IS AN EQUAL
OPPORTUNITY EMPLOYER (W6786)A

dkfz

Position in Classical and Molecular Cytogenetics

(headed by M. Schwab)

A position is available to study genetic contributions to human cancers. Our scientific activities address two subjects:

1. Identification and definition of genetic alterations in solid human cancers, in particular colon cancer and neuroblastoma.
2. Identification of the molecular basis of the regulation and function of the gene MYCN.

The successful candidate must have a Ph.D., preferencially at least two years of postdoctoral training and is expected to develop a vigorous research program addressing one of the two subjects, depending on his scientific interest.

The initial appointment will be for 2 years, salary is according to experience on the BAT scale. Applications should include a list of publications, a curriculum vitae and the names and addresses of two referees.

For details contact:
M. Schwab,
Deutsches Krebsforschungszentrum,
Institut für Experimentelle Pathologie,
Im Neuenheimer Feld 280,
D.6900 Heidelberg Phone: 06221–484 220 (W6798)A

UNIVERSITY OF LONDON INSTITUTE OF PSYCHIATRY

DEPARTMENT OF NEUROPATHOLOGY

AIDS RESEARCH RESEARCH WORKER

Applications are invited for the above post from suitably qualified scientists. The Research Worker is required to set up and maintain a national data base of laboratory, clinical and pathological information in relation to HIV infection and the nervous system. The Research Worker is expected to collaborate with basic and clinical neuroscientists. The candidate should preferably have some expertise in the use of data base and personal computers; further training can be obtained. The post will provide excellent opportunity for personal research.

The project is supported by the MRC for five years.

Salary is in the range £9,816–£12,381 pa plus £1,650 pa London Allowance.

Further information can be obtained from Professor P L Lantos on 01–703 5411 Ext 3272. A curriculum vitae, with the names of two referees, should be sent to the **Personnel office, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF.** Closing date 28 December 1989. (2243)A

M.I.T. Department of Biology

Faculty Positions in Genetics and Development

Candidates are sought for two tenure track positions at the level of Assistant Professor. Candidates should have significant research experience in either Genetics or Development. The responsibilities of this position include conduct of an active research program and participation in the teaching program of the Department.

Applicants should send a curriculum vitae and a summary of proposed research, and arrange to have letters sent from three scientists who can provide an evaluation of the candidate's accomplishments and future potential for both research and teaching. Applications and letters should be sent to:

Chairman, Genetics and Development Search Committee
c/o Room 56-511, Dept. of Biology
Massachusetts Institute of Technology
77 Massachusetts Avenue
Cambridge, Massachusetts 02139

M.I.T. is An Equal Opportunity/Affirmative Action Employer

(NW4698)A

MICROBIOLOGIST ASSISTANT, ASSOCIATE, OR PROFESSOR

The Department of Microbiology, University of California at Davis invites applications for an 11-month, tenure-track, open rank position from candidates who have demonstrated excellence in microbiological research. The candidate will join a strong, supportive and growing Department with strength in diversity, physiology and genetics. Teaching responsibilities include graduate and/or undergraduate courses in bacterial physiology and genetics. Please send a curriculum vitae, selected publications, a statement of proposed research, a description of teaching experience and interests, and the names of three references to: **Dr. J. C. Meeks, Chair, Physiology/Genetics Search Committee, Department of Microbiology, University of California, Davis, CA 95616.**

The position will remain open until filled; however, to be assured of consideration, applications should be received by February 1, 1990.

The University of California is an Affirmative Action/Equal Opportunity Employer. Women and Minorities are encouraged to apply. (NW4597)A

DANA-FARBER CANCER INSTITUTE HARVARD MEDICAL SCHOOL

Professor of Biological Chemistry and Molecular Pharmacology

Dana-Farber Cancer Institute (DFCI) and Harvard Medical School are seeking a Professor of Biological Chemistry and Molecular Pharmacology with a primary research interest in the molecular biology of gene regulation as it relates to the differentiation of mammalian somatic cells. The candidate should be a distinguished investigator, and will become a member of the new Division of Cell and Molecular Biology at the DFCI. The successful candidate will be expected to carry out a strong basic science research program, and participate in the teaching of medical and graduate students in the fields of cell and molecular biology.

Harvard University is committed to increasing representation of women and minority members among its faculty and particularly encourages applications from such candidates.

Candidates should send curriculum vitae, statement of research interests, and names of three references to:

DAVID M. LIVINGSTON, M.D., PROFESSOR OF MEDICINE, DANA-FARBER CANCER INSTITUTE, 44 BINNEY STREET, BOSTON, MA 02115.

Harvard Medical School and Dana-Farbar Cancer Institute are Affirmative Action-Equal Opportunity Employers. (NW4681)A

POSTDOCTORAL POSITION AT LOMA LINDA UNIVERSITY IN SOUTHERN CALIFORNIA

Ph.D. in one of the Biological Sciences is sought to conduct research on in vivo studies of bone metabolism in a large multi-disciplinary laboratory. Position has potential to develop into a permanent position (i.e., 3 years of successful performance will qualify the candidate for a tenure-track academic position). Experience in bone metabolism and U.S. citizenship is desirable but not required. Salary and Benefits Competitive.

Please send C.V., transcripts and 3 letters of recommendation to: **David J. Baylink, M.D., Chief, Mineral Metabolism (151), J.L. Pettis Veterans Hospital, 11201 Benton Street, Loma Linda, CA 92357.**

(NW4674)A

Institute for Small Animal Science P.O. Box 280, 3100 Celle, F.R.G.

Molecularbiologist Postdoctoral Position

Applications are invited from suitably qualified Postdoctoral Molecularbiologist to work in the Research Unit Physiology on an exciting project on the Ontogeny and Regulation of gene expression in the prenatal neuroendocrine brain. The position is funded by the German Research Foundation (DFG) for initially two years (extension for another two years possible) and is immediately available.

Please send CV, list of publications and the names of two referees to Dr. R. Grossmann at the above address by 31st December 1989. (W6791)A

A leading pharmaceutical Company
is seeking a

HEAD OF THE DEPARTMENT OF DOWNSTREAM PROCESSING PROTEIN CHEMISTRY

for its R. & D. Department
based in Milano — Italy

We require a Scientist (PhD level) to lead a group of 16–18 scientists and technicians involved in the purification of a number of recombinant proteins, scaling up of these procedures and eventually pilot production.

The ideal candidate will have at least 2—3 years post doctoral experience in an academic or industrial environment.

Spoken English is an important requirement.

We offer a competitive salary and encourage career development.

Sintex

Candidates should send a C.V **with Ref. A—1756 evidenced on the envelope** to: SINTEX S.r.l. – 20146 MILANO – Via Frua 22 – ITALY (W6792)A

UNIVERSITY OF STIRLING
BIOLOGICAL AND MOLECULAR SCIENCES
POST DOCTORAL FELLOW

A position is available from 1 March 1990, to work on a three-year AFRC funded project concerned with the molecular biology of anther development. Salary will be on the Research IA scale £10,458 to £16,665 pa.

Applications are invited from biochemists/molecular biologists, which should include full c.v. and names and addresses of two referees. These should be sent by 20 December 1989 to **Dr M J North, Department of Biological and Molecular Sciences, Stirling University, Stirling FK9 4LA, tel no 0786 73171 ext 2083,** from whom further particulars are available on request. (2260)A

UM

The University of Manitoba
Faculty of Science

MOLECULAR BIOLOGIST

The department of zoology, University of Manitoba has a tenure track position for a Molecular Biologist to start September, 1990. Salary is dependent on qualifications and experience. Applicants must have a Ph.D. with postdoctoral experience in development, evolution, or genetics. Promising applicants from other areas of metazoan molecular biology are not excluded. Responsibilities include undergraduate and graduate teaching and developing an independent, vigorous research program. Start-up support is available and opportunities exist for collaborative research. Both women and men are encouraged to apply. In accordance with Canadian Immigration requirements, priority will be given to Canadian citizens and permanent residents. Applicants must submit a c.v., description of research interests, representative reprints and names of at least 3 references by March 31 to: **Dr. J.H. Gee, Zoology Dept., Univ. of Manitoba, Winnipeg, MB Canada R3T 2N2.** (NW4700)A

SIMON FRASER UNIVERSITY
CHAIR OF THE DEPARTMENT OF BIOLOGICAL SCIENCES

Applications and nominations are sought for the position of Chair of the Department of Biological Sciences at Simon Fraser University. The Department has 35 faculty members whose teaching and research interests cover all the modern areas of Biological Sciences. There is a graduate enrolment of some 160 students, and external research grant support in 1988/89 was $2.4 million.

A Departmental Chair appointment is for up to five years and is renewable; the salary is negotiable. The appointment would normally carry tenure. Candidates should have an excellent record in research and a demonstrated ability as an academic leader.

Applications or nominations should be forwarded, by January 15, 1990, to the **Dean of Science, Simon Fraser University, Burnaby, B.C. V5A 1S6.**

This advertisement is directed to people who are eligible for employment in Canada at the time of application. S.F.U. offers equal employment opportunities to qualified applicants. (NW4626)A

UNIVERSITY OF CAMBRIDGE
DEPARTMENT OF PHARMACOLOGY

Rhone-Poulenc Post-Doctoral Research Fellowship in Computer-aided Drug Design

Applications are invited for the post of Rhone-Poulenc Research Fellow to pursue research in the field of molecular matching. The project is designed to identify sub-regions of similarity in electrostatic potential on the surfaces of dissimilar molecules and explore this topographical relationship with affinity and biological action. The applicant should have a Ph.D. and experience in computing in a shared mainframe environment. An Evans and Sutherland PS390 graphics computer is available. The project is under the direction of Dr P.M. Dean.

The appointment, which will date from 1st October 1989, will be for 2 years. Salary scale £10,460–14,500. Applications, which should include a C.V. and the names of two referees, should be sent as soon as possible to **Dr P.M. Dean, Department of Pharmacology, University of Cambridge, Tennis Court Road, Cambridge CB2 1QJ.** (2255)A

POSTDOCTORAL POSITION
AT THE DANA-FARBER CANCER INSTITUTE, HARVARD MEDICAL SCHOOL
Molecular Basis of Recognition by T cell Receptor Gamma-Delta

Position available to study molecular basis of antigen recognition by human gamma-delta T cell receptor. Work involves derivation and characterization of antigen-specific gamma-delta T cell clones, cloning, sequencing and transfection of T cell receptor genes, and in vitro site-directed mutagenesis of T cell receptor genes to delineate the residues involved in antigen recognition. Position carries an appointment at Harvard Medical School. Please send curriculum vitae and names of 3 references to **Dr. Hamid Band, Laboratory of Immunochemistry, Mayer 640, Dana-Farber Cancer Institute, 44 Binney Street, Boston, MA 02115, or call at (617) 732-3085.**

An Affirmative Action/Equal Opportunity Employer. (NW4680)A

POST DOCTORAL POSITIONS AVAILABLE IN MOLECULAR ENDOCRINOLOGY

Individuals with strong backgrounds in molecular and/or cellular biology are invited to apply for post doctoral positions in a leading laboratory involved in the investigation of the biosynthetic and regulatory mechanisms in the production of the islet hormones-insulin, amylin, glucagon and others. Areas of concentration include islet prohormone conversion mechanisms, regulation of islet hormone gene expression, expression and role of ion channels in islet cells, insulin receptor signal transduction mechanisms, studies on islet hormone gene evolution and on mutant insulin and insulin receptor genes in human populations.

Contact:

Donald F. Steiner, M.D., Senior Investigator
Howard Hughes Medical Institute – N214
Box 23
The University of Chicago
5841 S. Maryland Avenue
Chicago, Illinois 60637 (NW4690)A

Wayne State University

FACULTY POSITION IN NMR

The Department of Biochemistry is seeking applicants to fill a tenure-track position. Appointments at any level will be considered. Applicants should have experience in high resolution NMR determination of structure of proteins or other macromolecules, with appropriate graduate and postdoctoral training. Interested individuals should send curriculum vitae, outline of research interests, and the names of three references to: **Barry P. Rosen, Professor and Chairman, Department of Biochemistry, Wayne State University, School of Medicine, 540 East Canfield Avenue, Detroit, MI 48201.**

Wayne State University is an Equal Opportunity/ Affirmative Action Employer. Women and minorities are encouraged to apply. (NW4668)A

ASSISTANT PROFESSOR

Applications are invited for a position at the level of assistant professor (tenure track) in the Department of Chemistry at Stanford University, with primary research and teaching interests in the general area of inorganic chemistry. Applicants must be strongly motivated toward creative research and have a commitment to teaching at the undergraduate and graduate levels. In applying, please send curriculum vitae, list of publications, a brief statement of research interests (three pages or less) and, in addition, have three letters of recommendation sent to **Professor Harden M. McConnell, Chairman, Department of Chemistry, Stanford University, Stanford, California 94305.**

Stanford is committed to Equal Opportunity through Affirmative Action in employment, and we are especially eager to identify minority persons and women with appropriate qualifications. Applications should be received by January 15, 1990. (NW4629)A

MEDICAL RESEARCH COUNCIL
NATIONAL INSTITUTE FOR MEDICAL RESEARCH
LABORATORY OF GENE STRUCTURE AND EXPRESSION

POSTDOCTORAL SCIENTIST

Applications are invited for the post of postdoctoral scientist to work with Dr Elaine Dzierzak in the Laboratory of Gene Structure and Expression (Head: Dr Frank Grosveld). The AIDs-Directed research programme is focussed on intracellular molecular ablation strategies and involves the use of recombinant retroviral vectors. Candidates are expected to have experience in recombinant DNA techniques and mammalian cell culture. A strong background in mammalian gene expression would be favoured.

The position will be tenable for 3 years. The salary will be in the range £12,381 to £20,469 (starting salary dependent on experience) plus £1,650 per annum London Allowance. MRC Pension Scheme option.

Applications including a curriculum vitae, list of publications and the names and addresses of two professional referees should be sent to Mr C R Russell, Administrative Manager, National Institute for Medical Research, The Ridgeway, Mill Hill, London, NW7 1AA, not later than 29th December 1989 quoting reference number GGSE/ADP/PD. Informal enquiries about the nature of the work may be made to Dr Elaine Dzierzak on ext. 2095.

The MRC is an Equal Opportunities Employer. (2247)A

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— EVERY WEEK

ASSISTANT PROFESSOR

Department of Medicine, Indiana University School of Medicine, with possibility of a joint appointment in microbiology and immunology. Initial research would focus on genetics of virulence factors, including antibiotic resistance, in chlamydiae. A strong background in molecular biology is required. Interested parties reply with a curriculum vitae to: **Robert B. Jones, M.D., Ph.D., Department of Medicine, Room 435 Emerson Hall, 545 Barnhill Dr. Indianapolis, IN 46202-5124.** (NW4682)A

RESEARCH SCIENTIST

($A31,525-$A38,567)

CSIRO DIVISION OF BIOTECHNOLOGY
LABORATORY FOR MOLECULAR BIOLOGY
SYDNEY, AUSTRALIA

THE JOB: The appointee will join a team investigating the interaction of cells with natural extracellular matrix components and with synthetic surfaces. The research aims to use an understanding of extracellular matrix structure and function for the rational design at a molecular level of biomaterials with improved biocompatibility. The duties will include the evaluation of surfaces produced by RF plasma techniques for biocompatibility and blood compatibility, and participation in the design of novel composites of synthetic polymers combined with biological factors for biomaterial applications.

THE PERSON: Applicants should hold a Ph.D. or equivalent qualification in cell biology/biochemistry/anatomy or histology or related discipline. An applicant having experience in one or more of the following areas would be preferred:

- cell biology of tissue structure and function
- control of cell phenotype by trophic factors and extracellular matrix components
- biocompatibility and haemocompatibility of biomaterials
- mechanisms of wound healing responses.

CONDITIONS: Indefinite appointment with Australian Government Superannuation.

MORE INFORMATION: Prospective applicants are invited to contact Dr Jack Steele or Dr Anne Underwood in writing to the address below, by fax to 888 9271 or 61-2-886-4805 or by phone on 61-2-886-4888 for further information. Ms Maree O'Neill may be contacted on these same numbers to provide selection criteria.

APPLICATIONS: Applications quoting reference No. PG1/57 should be submitted by 18th December 1989. Wherever possible they should be framed against the selection criteria and should state relevant personal particulars, including details of qualifications and experience. All applicants should also nominate at least two professional referees. Applications may be faxed to either fax number above or posted to:

The Chief,
CSIRO Division of Biotechnology,
PO Box 184,
North Ryde, NSW 2113,
Australia.

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(W6802)A

POSTDOCTORAL AND RESEARCH ASSOCIATE POSITIONS

Positions are available in studies of gene expression and regulation in inborn errors of metabolism, including (1) disorders of biotin-dependent carboxylases, (2) lysosomal storage diseases, and (3) development of disease models mediated by homologous recombination. Salaries commensurate with experience. Send curriculum vitae and names of three references to:

Dr. R. Gravel,
McGill University-Montreal Children's Hospital
Research Institute,
2300 Tupper Street,
Montreal, Quebec, Canada, H3H 1P3.

(NW4702)A

ASSISTANT RESEARCH SCIENTIST IN X-RAY CRYSTALLOGRAPHY

The position will involve: equipment development and maintenance, program development, crystal growth and data collection, and structure solutions. Excellent x-ray crystallographic equipment exists and a number of interesting proteins are under study. Applications from scientists with background in small molecular crystallography are welcome. Review of applications will begin January 15, 1990 but applications will be accepted until the position is filled. Salary commensurate with experience. This is a state-funded, continuing eligible position. Candidates are eligible to secure grant funding for their research.

Minority and women candidates are urged to apply.

Submit a CV and three letters of reference to: **Dr. William Montfort, Dept of Biochemistry, Biosciences West, University of Arizona, Tucson, AZ 85721.**

An Equal Opportunity/Affirmative Action/Title IX/Section 504 Employer. (NW4676)A

The University of Bayreuth announces three tenured positions in the newly formed Bayreuth Institute of Terrestrial Ecosystem Research (BITÖK):

PROFESSOR OF ECOSYSTEM PLANT ECOLOGY
PROFESSOR OF SOIL MICROBIOLOGY
PROFESSOR OF SOIL SCIENCES

BITÖK was formed in 1989 with the aim to study the processes governing ecosystem structure and function. Specifically, the program's main emphasis is to understand and quantify temporal and spatial variations in the supply and demand of carbon and nutrients in ecosystems. The research in BITÖK is cooperative with that of the faculty of Biology, Chemistry and Geosciences at the University of Bayreuth. The institute is funded by a grant from the German Federal Minister of Science and Technology (BMFT). At its completion, BITÖK will contain departments of ecosystem plant ecology, soil science/soil physics, soil microbiology, hydrogeology, zoology, climatology and ecological modelling. In addition to these departments the institute will contain a technical group which will provide support including computer expertise, electronical and mechanical workshops and analytical chemistry.

Description of position: This is a newly established full-time, 12 months, faculty appointment with tenure and professional responsibilities at BITÖK. Individual professors in BITÖK are expected to cooperate in an integrated research program in the context of terrestrial ecosystems.

Qualifications: Applicants must have a Ph.D. degree in the respective field. Applicants who have not completed their habilitation (German system) or are not yet tenured would be required to demonstrate an outstanding research and teaching experience in the field. A great weight in hiring will be given to an ability for interdisciplinary research. In the initial phase of BITÖK the position responsibilities include establishing, in cooperation with other departments, scientific programs and the necessary laboratory capacities. The responsibility also includes teaching. The University of Bayreuth is an equal opportunity employer.

Salary will be competitive and paid according to the C4 level (a German equivalent of a full professor in the American system).

Support: The research program is supported by BITÖK with research assistants, technicians and a research budget.

Applications must include a letter of research perspectives, a curriculum vitae (including a complete list of publications, and an up-to-date short description of research and teaching experience), and reprints of important publications. The applications should be addressed to:

Dean of the Faculty of Biology, Chemistry and Geosciences,
University of Bayreuth,
POB 10 12 51,
8580 Bayreuth,
Federal Republic of Germany

Official closing date for applications is January 25, 1990.

Information: contact the Bayreuth Institute of Terrestrial Ecosystem Research,
Scientific Secretary, POB 10 12 51 D-8580 Bayreuth, FRG
(University of Bayreuth — FAX: FRG – 921 – 6 48 89)

(W6806)A

NEUROSCIENCE DEPARTMENT

Roche Institute of Molecular Biology

Cellular/Molecular Neuroscientist, Assistant Professor Level

The department currently has well developed interests in inter- and intracellular signalling mechanisms and gene expression/regulation in adult and developing nervous systems. Individual will be expected to establish an independent basic research program in one of these general areas of neuroscience. The Institute provides investigator salary and generous support for supplies, equipment and personnel. Applicants should submit resume and an outline of research by February 1st, 1990, to: **Dr. John A. Connor, Head, Department of Neuroscience, Roche Institute of Molecular Biology, 340 Kingsland Street, Nutley, New Jersey 07110.** We are an equal opportunity employer.

Roche Institute of Molecular Biology
ROCHE Hoffmann-La Roche Inc

(NW4688)A

INSTITUTE FOR
ANIMAL HEALTH

VIROLOGIST — MOLECULAR BIOLOGIST — BIOCHEMIST

Postdoctoral position (2–3 yr) available at the Pirbright Laboratory to work with a group engaged in basic research on the genetics and molecular biology of FOOT-AND-MOUTH DISEASE VIRUS (FMDV). Ours is an active, rapidly expanding group, which benefits from strong collaborative links with Universities. The group forms part of the DIVISION OF MOLECULAR BIOLOGY, which comprises some 30 scientists, PhD students and assistants.

The project is to engineer new antigenic sites into FMDV, a small RNA virus whose crystal structure was recently solved. The successful candidate will be seeking a challenging research project in the area of protein engineering/chimaeric viruses. He/she will have a PhD and experience in some area of molecular biology or a related discipline.

PIRBRIGHT LABORATORY, located near Guildford and Woking, is the UK centre for the study of exotic viruses of domestic animals, and is home to the World, and European, Reference Laboratories for FMDV.

Salary between £10,678 and £18,864 according to age and experience, plus non-contributory pension scheme. HOUSING available.

ENQUIRIES to Dr Andrew King (0483232441). To apply, please forward CV and names of two referees to **The Pirbright Laboratory, IAH, Ash Road, Pirbright, Woking GU24 0NF.**

Closing date: 22nd December 1989.

The AFRC is an equal opportunities employer. (2294)E

IMMUNEX RESEARCH AND DEVELOPMENT CORPORATION, a Seattle-based biopharmaceutical company committed to research and development for treating diseases related to malfunctions in the immune system, is currently recruiting for:

Staff Scientists and Postdoctoral Fellows

With experience in the following areas:

Process Development/ Biochemical Engineering

Successful candidates will work on purification, scale-up, and refolding of recombinant proteins expressed in microbial and mammalian cell hosts. Additional positions are available for work on protein characterization and formulation. A background in biochemical engineering, biochemistry or protein chemistry is preferred.

Experimental Hematology

Staff Scientist needed to contribute to experimental hematology program. Researcher needed for development of in vitro and in vivo models of hematopoiesis. Particular emphasis will be placed on the establishment of assays having the capacity to detect novel hematopoietic growth factors.

Immunology

Scientific researcher needed with expertise in culturing of monocytes/macrophages and assessment of their activation. Expertise in dealing with human cells a plus; experience with cytokines desired.

Immunex Research and Development Corporation offers excellent opportunities for career development as well as competitive salaries and an excellent benefits package. For consideration, please submit C.V., names of three references, and list of publications to: **Immunex Research and Development Corporation, Human Resources Director, 51 University Street, Seattle, WA 98101.** EOE.

(NW4675)A

immunex

Technische Universität Braunschweig

An der *Naturwissenschaftlichen Fakultät* ist im Rahmen eines gezielten Ausbaus der Biowissenschaften sofort folgende Stelle erstmals zu besetzen:

eine Professur (C4) für Zellbiologie/Molekularbiologie

Von dem künftigen Stelleninhaber wird erwartet, dass er den im Aufbau befindlichen Forschungsschwerpunkt Biotechnologie durch seine Forschung prägend mitbestimmt und aktiv an dem neu eingerichteten grundständigen Studiengang Biotechnologie und dem Schwerpunktstudium Bioverfahrenstechnik mitwirkt. Der Stelleninhaber wird über eine solide personelle und sächliche Grundausstattung verfügen und hervorragende Arbeitsmöglichkeiten im Biozentrum vorfinden. Durch die enge Zusammenarbeit auf dem Gebiet der Biotechnologie zwischen der Technischen Universität und der Grossforschungseinrichtung GBF (Gesellschaft für Biotechnologische Forschung mbH) bestehen darüber hinaus attraktive Möglichkeiten für Kooperationen.

Die allgemeinen Einstellungsvoraussetzungen für Professoren sind in §56 des Niedersächsischen Hochschulgesetzes geregelt, dessen Text auf Anforderung zugesandt wird.

Bewerbungen sind innerhalb von 8 Wochen nach Erscheinen dieser Anzeige an den **Vorsitzenden der Naturwissenschaftlichen Fakultät der TU Braunschweig, Professor Dr. H. Brass, Pockelsstrasse 14, D-3300 Braunschweig,** zu richten, der auf Anfrage auch über weitere Einzelheiten Auskunft gibt. (W6807)A

PLANT BIOLOGY RESEARCH FELLOWSHIPS

SAMUEL ROBERTS NOBLE FOUNDATION and THE SALK INSTITUTE JOINT PROGRAM

Applications are now being accepted for two Noble Foundation Fellowships for post-doctoral research in Plant Molecular Biology. These Fellowships are a key element in a collaborative program between the Plant Biology Division, Noble Foundation, Ardmore, Oklahoma, directed by Dr. Richard A. Dixon, and the Plant Biology Laboratory, The Salk Institute for Biological Studies, La Jolla, California, directed by Dr. Christopher J. Lamb.

Research topics include biochemical, molecular and genetic analysis of signal transduction pathways for environmental and developmental regulations of plant gene expression. The Fellowships are for three years beginning September 1990. Eighteen months will be spent in each laboratory.

Please send resume and three letters of recommendation to Dr. C.J. Lamb, The Salk Institute for Biological Studies, P.O. Box 85800, San Diego, CA 92138. Telephone (619) 453-4100, x106.

Equal Opportunity Employer M/F/H/V (NW4710)A

SENSORY SCIENTIST

Research Scientist — Senior Research Scientist

$A31,525-$A45,699

SENSORY RESEARCH CENTRE
CSIRO DIVISION OF FOOD PROCESSING
SYDNEY, AUSTRALIA

The CSIRO Division of Food Processing has maintained a strong interest in sensory research for 20 years and its achievements have earned it a world wide reputation for excellence in gustation and olfaction. In 1987 the Division established the Sensory Research Centre to consolidate its sensory research, assist the Australian food industry to improve product quality and to become a centre of expertise on Asian food preferences. The Centre has established a successful sensory evaluation research service for the food industry and has recently received substantial funds for a major study of sensory responses of Asian populations. The Centre is currently planning a study located in Japan. Within the next two years it is expected that the Centre will move into a new building at the Sydney site, the function of which is to support its Australian and Asian projects. The Division of Food Processing is committed to assisting development of Australian food exports through its excellence in scientific activity.

THE JOB: Conduct fundamental studies on the sense of taste with the objective of understanding the underlying factors which determine food acceptance. The studies could embrace research on psychophysical measurement techniques, complex taste stimuli; integration of odour-taste responses, psychophysiological approaches, and the effects of age, experience, culture and disease on taste and food acceptance. The successful applicant will be expected to participate in the other activities of the Sensory Research Centre which will involve sharing responsibility with the two Principals of the Centre for the supervision of short term sensory evaluation contract research with the food industry and studies of cross cultural food preferences of Asian populations. In view of the blend of long and short term research work this challenging position will entail, a creative, entrepreneurial scientist is sought. A higher salary can be negotiated for an outstanding candidate.

THE PERSON: Applications are invited from scientists with a record of research in sensory perception. A highly motivated person is sought who is interested in establishing a reputation for excellence in the field of taste research and in applying new knowledge to practical areas of sensory perception.

QUALIFICATIONS: A Ph.D or equivalent in the behavioural or physiological sciences or related scientific discipline with experience in sensory perception.

TENURE: Appointment will be for an indefinite period with Australian Government superannuation benefits.

THE DIVISION: The Food Research Laboratory, Division of Food Processing, is situated at North Ryde, some 12km from the city of Sydney. Its research programmes reflect the Division's objectives which are to develop ways to handle, process and add value to foods, to improve efficiency, quality and food safety and to minimize or utilize waste. Areas of activity at the Laboratory include sensory studies, separation processes, process engineering, preservation technology, microbial physiology, food spoilage, and food pathogens. An Industry Initiatives Group deals with liaison, communications and general publications.

MORE INFORMATION: Prospective applicants are invited to write or phone Dr David Laing or Dr Graham Bell on 61 02 887 8333 or by fax on 61 02 887 3107 for further information. Copies of the detailed job description and selection criteria are available on request.

APPLICATIONS: Applications should be submitted by 22 January 1990 and should quote the reference number (FD89/14). They should be framed against the selection critieria and should provide details of relevant personal particulars including qualifications and experience. Applicants should nominate at least two professional referees and address their applications to:

The Chief,
CSIRO Division of
Food Processing
PO Box 52,
North Ryde, NSW 2113,
Australia.

or they may be faxed to 61 02 887 3107.

CSIRO AUSTRALIA

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER (W6808)A

LUDWIG INSTITUTE FOR CANCER RESEARCH

St Mary's Branch

A postdoctoral position will become available in the Epstein-Barr virus group from February 1990. The post might particularly interest candidates with an interest in the biology of B cell growth control, control of gene expression in response to cell differentiation or genetic approaches to analysing viral functions. The Institute offers excellent working conditions in newly refurbished laboratories in central London.

Applications including CV and the names of three referees should be sent to **Dr. P.J. Farrell, Ludwig Institute for Cancer Research, St Mary's Hospital Medical School, Norfolk Place, Paddington, London W2 1PG.** Tel: 01-724 5522. (2193)A

Wellcome Diagnostics

Post Doctorates

Vacancies in Virology

£15,000 - £23,000 **Beckenham, Kent**

Wellcome Diagnostics develops, manufactures and markets a wide range of medical diagnostic kits for use in hospital laboratories and blood banks. Rapid expansion demands that we look for Post Doctoral Scientists to work in this important area of research.

Allowing you the freedom to approach problems in a creative and innovative way will enable you to complete research projects using expert scientific knowledge and the latest technology within a well funded environment.

We are looking for 2 scientists in the following area:

As a committed Enzyme Immunoassayist you will apply your experience in the fields of retroviruses, hepatitis and other virally transmitted diseases to invent and develop immunoassays.

A knowledge of the safe handling of pathogens is essential; you must have experience in micro-titre plate based assays and be prepared to work to ensure the complete development of assays through to Production.

The other post requires new and creative ideas in the field of immunoassays with a view to making novel formats commercially viable over the next couple of years. You will also provide protein chemical support for the current generation of immunoassays.

Self motivated and highly creative, you will probably be a chemically orientated biochemist or immunologist with some experience of peptide and recombinant protein chemistry. Your experience may have included enzyme modification or engineering but will definitely have included protein chemistry and possibly nucleic acid chemistry.

Scientific collaboration and publication of your work in respected journals will enable you to promote awareness of Wellcome Diagnostics.

Experience, maturity and the ability to make a valid contribution to research will be reflected by a highly competitive salary. In addition, you can look forward to a comprehensive range of benefits including 5 weeks holiday, pension and life assurance schemes, a subsidised restaurant and first-class sports and social facilities.

Please write with full career details, quoting reference number 91/182 to Susan Lark, Personnel Assistant, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent, BR3 3BS.

- **The Wellcome Foundation Ltd.**
- **Major International Pharmaceutical Group**
- **Research Centre at Beckenham**
- **Annual Turnover > £1,400m**
- **An Equal Opportunity Employer**

Wellcome

(2289)A

The Imperial Cancer Research Fund is one of the largest charity research institutions in Britain with wide ranging interests in fundamental, applied and clinical research.

Research Fellowship

A post-doctoral fellowship is available to work on novel gene organization and regulation in mammalian cells. The molecular mechanisms and cis and trans acting sequences involved in the control of gene expression and gene amplification will be investigated. Candidates should have experience in molecular biology and recombinant DNA technology. The award will be tenable for up to three years with a salary of **£14,800-£18,500** depending on experience.

For further details contact Dr. Mike Fried on 01-269 3297. Please send full curriculum vitae and names and addresses of three referees to the address below, quoting reference 34/R.

Scientific Officer/ Senior Scientific Officer

We require an SO/SSO to work with Dr P. Goodfellow in the Department of Human Molecular Genetics. The work involves the in vitro production and biochemical purification of proteins produced by genes located on the human Y chromosomes.

Degree or equivalent is required in a relevant subject (SO) with a minimum of 6 years working in a research laboratory (SSO). Experience in biochemical techniques and ability to work independently are both essential. Some experience in immunogenetics and molecular genetic techniques would be an advantage, but, is not essential. Ref: 41/R.

£11,200-£17,800

Scientific Officer/ Senior Scientific Officer

We are also looking for an SO/SSO to work at our Biochemical Regulatory Mechanisms department in WC2.

You will assist in analysing the second message (signal transduction) pathways and factors involved in the control of interferon-inducible gene expression and mechanisms by which the interferons inhabit virus replications and cell growth.

Degree or equivalent is required in a relevant subject (SO) with a minimum of 6 years working in a research laboratory (SSO). Experience in molecular biology, biochemistry and tissue culture would be advantageous. Ref: 42/R.

£11,200-£17,800

For further information and application form, please contact the Recruitment Officer,
Imperial Cancer
Research Fund,
Lincoln's Inn Fields,
London WC2A 3PX.
Tel: 01-242 0200 ext. 3357, quoting the appropriate reference.

SMOKING IS ACTIVELY DISCOURAGED.

Imperial Cancer
Research
Fund (2292)A

FACULTY POSITIONS

The Physics Department i
vites applications for tenur
track positions at the Assista
Professor level. It is expecte
that up to three positions w
be filled in the next year. Th
prime objective of the Depar
ment is to augment i
research strength in the area
of experimental elementa
particle and experimental co
densed matter physics. Hov
ever, exceptional candidate
in any field of physics ar
encouraged to apply.

Candidates should have a Ph.D
degree or equivalent, som
postdoctoral experience,
good research record and a
aptitude for undergraduate an
graduate teaching. The ap
pointments are subject to fina
budgetary approval.

The University of British Co
umbia is committed to th
Federal Government's em
ployment equity program an
encourages applications from
all qualified individuals. I
accordance with Canadia
immigration requirements
priority will be given to Cana
dian citizens and permaner
residents of Canada.

Applicants should submit
curriculum vitae and a state
ment of current research in
terests and future plans. They
should also arrange to have
three letters of reference sen
directly to: **Prof. B.G. Turrel**
Head, Department of Physics
The University of British Col
umbia, 6224 Agriculture Road
Vancouver, B.C. V6T 2A6.

The deadline for receipt o
applications is March 15th
1990. (NW4701)A

ASSISTANT PROFESSOR
MOLECULAR/CELLULAR NEUROBIOLOGY

The Department of Neurobiology and Physiology, Northwestern University invites applications and nominations for a tenure-track faculty position at the rank of Assistant Professor beginning the fall of 1990. Outstanding applicants working in any area of neuroscience will be considered, although candidates working in molecular or cellular neurobiology are particularly encouraged to apply. There are 21 faculty in the Department with research programs in sensory neurobiology, neuroendocrinology, development and plasticity. The Department is a central component of the newly formed Institute for Neuroscience consisting of over 70 faculty. Our Department and the Department of Biochemistry, Molecular Biology and Cell Biology form the life sciences division on the Evanston campus. Applicants should have a Ph.D. or M.D. and have completed postdoctoral training. Curriculum vitae, list of publications, a description of current and future research, and three letters of recommendation should be sent to: **Dr. Lawrence Pinto, Search Committee Chairman, Department of Neurobiology and Physiology, Northwestern University, 2153 Sheridan Road, Evanston, Il 60208.**

Northwestern University is an equal opportunity, affirmative action educator and employer. (NW4692)A

LANCASTER UNIVERSITY
Institute of Environmental and
Biological Sciences
Division of Biological Sciences

RESEARCH ASSOCIATE

Required to work with Dr R.I. Jones on a 3-year NERC-funded investigation into the "Role of phagotrophy in nutrient acquisition by phytoflagellates". Applicants should have, or expect to gain shortly, a PhD and preferably have a background in aquatic science or environmental microbiology, and experience of radioisotope work, electron microscopy and algal culture would be helpful.

Starting salary £10,458 on the Research range 1A (£10,458–16,665 pa).

Further details and application form (quoting reference L000755) available from **Personnel Services, University House, Bailrigg, Lancaster LA1 4YW** where applications (5 copies) should be received by 4 January, 1990. Tel: 0524 65201 ext. 2160. (2291)A

CARDIFF

SCHOOL OF PURE AND APPLIED BIOLOGY

RESEARCH ASSOCIATE (Fixed-Term 3 Years) AND STUDENTSHIP

A Research Associate with a PhD in evolutionary genetics, animal behaviour or a related discipline is required to work with Dr R K Butlin on mating patterns in a grasshopper hybrid zone.

Salary: £10458-£16665 per annum.

For further particulars and application form please write o **University of Wales Col- ege of Cardiff, PO Box 431, Cardiff CF1 1TA, quoting Ref 89/163.**

Closing Date: 5 January 1990.

A NERC Studentship in the same research area will be available from 1 October 1990. Enquiries should be directed to **Dr R K Butlin, School of Pure and Applied Biology, University of Wales College of Cardiff, PO Box 915, Cardiff CF1 3TL.**

(2281)A

Institute of Hydrology

GROUNDWATER HYDROLOGIST

Wallingford, Oxon

We are seeking one or more ground water hydrologists to join the Consulting Group of the Institute. This group undertakes applied projects for clients in the UK and overseas.

The post(s) will offer the opportunity to be involved in a range of research and applied studies in the field of water resources and environmental protection, mainly involving unconsolidated alluvial aquifers. Much of the work will be computer based and some competence with PC's and knowledge of the FORTRAN language would be an advantage. At least one candidate should be familiar with groundwater modelling techniques, which are an essential input to the majority of studies undertaken.

Applicants require a good honours degree in geology or related subject, and preferably a postgraduate qualification in hydrogeology. Some experience overseas would be an advantage but is not essential.

The appointment(s) will be for three years in the first instance. Salary will be in the range of **£10,678** to **£14,909** per annum, or **£13,254** to **£18,864** per annum, depending on qualifications and experience. Terms include a generous holiday allowance and non-contributory pension scheme.

The Institute of Hydrology is a component organisation of NERC.

Further information and application forms may be obtained from Sue Fenton, Institute of Hydrology, Wallingford, Oxon, OX10 8BB. Telephone 0491-38800. Closing date for applications is 28th December 1989.

Previous applicants need not apply.

The Natural Environment Research Council is an Equal Opportunities Employer.

Natural Environment Research Council

(2287)A

UNIVERSITY OF OXFORD

University Lectureship in Astrophysics in association with St. Catherine's College

Applications are invited for a University Lectureship in Astrophysics. The successful candidate will be offered a tutorial fellowship at St. Catherine's College. Some preference will be given to applicant's with research interests in extragalactic observational astronomy, but candidates from all branches of astrophysics are encouraged to apply.

Further particulars of the lectureship and of the college fellowship may be obtained from **Prof. G.P. Efstathiou, Department of Astrophysics, Keble Road, Oxford, OX1 3RH,** to whom a curriculum vitae should be submitted by 31st January 1990. Applcants should arrange for letters from three referees to reach the Department by the closing date.

(2293)A

The University is an Equal Opportunity Employer

PEPTIDE TECHNOLOGY LTD/CSIRO

(Molecular Biologist/Protein Chemist)

Oxidative radicals in sunlight induced damage and oncogenesis in skin.

Peptide Technology Ltd. is a Sydney-based biotechnology company with research and production facilities in Copenhagen (Carlbiotech A/S) and in Sydney. In association with collaborating institutions both in Australia and in Europe it is developing novel therapeutic products for use both in human health-care and in animal production. Current R & D programmes include the development of treatments for inflammatory conditions, cancer and certain immunological disorders, as well as enhancement of growth and fertility modification in the veterinary product area.

The Company requires an energetic and self-motivated scientist to study the significance of and prevention of free radical damage in skin cancer and aging in a joint project with CSIRO. The successful applicant will have a background in DNA or protein chemistry; molecular or cell biology.

The successful applicant will work in the Company's Biomedical Research laboratories at the Laboratory for Molecular Biology of the CSiRO Division of Biotechnology, N. Ryde, Sydney in association with a number of other scientists working in similar or complementary fields including Drs. R. Holliday FRS, G.Grigg, R. Aston, H. Drew and A. Underwood.

Starting salary will be between AUS $31,500 — $38,500 depending on qualification and experience. Other benefits include a generous superannuation scheme and share options.

Please write with full C.V. to the **Operations Manager, Peptide Technology Ltd., PO Box 444, Dee Why, NSW, 2099 Australia,** or Fax to 61 2 805 1630.

(W6800)A

FACULTY POSITION IN NEUROBIOLOGY

EUROBIOLOGY RESEARCH CENTER
UNIVERSITY OF ALABAMA AT BIRMINGHAM

e to continued expansion of the NRC in the B School of Medicine, a new tenure track sition in **MOLECULAR NEUROBIOLOGY** is ailable. The appointment will be at the **SISTANT** OR **ASSOCIATE PROFESSOR** level, mmensurate with experience. The successful ndidate will be expected to conduct an active, tramurally funded research program related to pects of synaptic function including normal nsmission, plasticity or development. Particu- consideration will be given to individuals ing contemporary tools that afford insight into NE EXPRESSION AND REGULATION. The ap- intment is on a 12 month basis and includes tionally competitive salary, newly revovated ace and start-up funds. The NRC is currently mposed of 16 faculty members with actively nded research programs in synaptic and mem- ane biophysics, molecular bioloigy of synaptic ansmission, neuroendocrinology, peptide odulation of neural rhythmicity, neuroem- yology and neuron/glia interaction in develop- ent and regeneration. Access to shared facili- es including a premiere neuronal image analy- s center and super computer network are avail- le in the NRC. UAB is a major biomedical re- arch center with thriving graduate programs in urobiology, Immunology and Molecular Biol- gy and is located in an economically favorable d culturally vibrant community. Applicants ould send curriculum vitae, statement of re- earch interests and have three letters of refer- nce sent by January 28th to: **Micheal J. Fried- nder, Director, Neurobiology Research Center, niversity of Alabama at Birmingham, Birming- am, Alabama, 35294.** *UAB is an affirmative ction/equal opportunity employer.* (NW4678)A

NA Library Availability

National Laboratory Gene Library Project

he Department of Energy National Labora- ries at Los Alamos and Livermore are currently onstructing large-insert lambda and cosmid varies for each human chromosome. The two boratories are seeking proposals from in- estigators who are interested in participating in ne characterization of these libraries on a col- borative and unfunded basis. Many types of haracterization data are needed, and the incor- oration of library usage into ongoing research vill provide useful information.

his is a competitive program; only a small umber of the best proposals will receive either hage and/or cosmid libraries at no cost. Distribution worldwide will take place only after his characterization phase has been suc- essfully accomplished. Partial digest libraries n both phage and cosmid vectors are available urrently (or early 1990) for chromosome 4, 5, 8 and 17 (from Los Alamos) and chromosomes 11, 21, 22, and Y (from Livermore). The deadline for ubmission of a short proposal is Monday, January 29, 1990.

Vrite to the appropriate laboratory, Larry L. Deaven, Life Sciences Division, Los Alamos Na- ional Laboratory, Los Alamos, NM 87545, fax: 505) 665-3024, or Marvin A. Van Dilla, Biomedical Sciences Division, Lawrence Liver- more National Laboratory, Livermore, CA 94551, ax: (415) 423-3608, for proposal forms.

University of California
Lawrence Livermore National Laboratory

(NW4708)A

Research Professionals in Biotechnology

ImmunoGen is a rapidly growing biotechnology company dedicated to the development of immunotoxins and other cancer therapeutics. We are seeking talented scientists to join us in exploring new approaches to human disease. Research positions are available at Ph.D., M.S. and B.S. levels in our expanding research programs. Candidates should enjoy taking the initiative in solving scientific problems to achieve near-term and long-term goals.

STAFF SCIENTISTS IN BIOCHEMISTRY

Candidates should have a Ph.D. in Biochemistry or related disciplines, with a strong research background in biochemistry. People with a proven record of realizing ideas through developing independent research projects, such as group leaders or junior faculty, would be strong candidates.

STAFF SCIENTISTS IN CELL BIOLOGY

Candidates should hold a Ph.D. in cell biology, immunology or related disciplines and have a strong research background and publication record in mammalian cell biology.

STAFF SCIENTIST IN MOLECULAR BIOLOGY

Our newly established molecular biology laboratory is developing a research program to include among other projects cloning, characterization and expression of cell surface antigens, construction, mutagenesis and expression of chimeric proteins. Qualified candidates should possess a Ph.D. in molecular biology and have a strong background in molecular cloning methods, cDNA library construction and screening, and DNA sequencing.

RESEARCH ASSOCIATES

A number of opportunities exist for associate scientists at the B.S. and M.S. level in cell biology, molecular biology, biochemistry and organic chemistry. Research areas include characterization and study of human cell lines, *in vitro* evaluation of immunotherapies for cancer and other diseases, DNA cloning and sequencing, drug synthesis and immunoconjugate construction.

ImmunoGen offers an excellent salary and benefits package including 100% paid Health and Dental Insurance and Tuition Assistance. The company is located in a new, fully equipped, state-of-the-art laboratory in Cambridge, midway between the campuses of HARVARD and MIT.

Please send a detailed resume including three references and a statement describing your scientific interests and career goals. All responses will be acknowledged. Reply to: ***Jeanne Horgan, Personnel Department, ImmunoGen, Inc., 148 Sidney Street, Cambridge, MA 02139.***

IMMUNOGEN, INC.

(NW4684)A

An Equal Opportunity Employer

INSTITUTE OF CHILD HEALTH
University of London
Leukaemia Research Fund Centre for Childhood Leukaemia
Department of Haematology and Oncology

CYTOGENETICIST

Applications are invited from candidates with experience in human cytogenetics for the above post in this expanding department. The person appointed will be required to assist in the processing and cytogenetic analysis of bone marrow and peripheral blood samples from children with leukaemia and other haematological disorders and to participate in research projects.

Salary on Probationary Biochemist scale £9,815-£12,340, including London allowance.

Informal enquiries to Dr. B.R. Reeves, 242 9789 ext. 2314, or Prof. J.M. Chessels, ext. 2318.

Further particulars are available from the **Personnel Assistant, 30 Guilford Street, London WC1N 1EH (tel: 01-242 9789 ext 2650)** to whom completed applications (3 copies) and names of two referees, should be sent as soon as possible. (2256)A

UNIVERSITY OF WALES COLLEGE OF MEDICINE
DEPARTMENT OF SURGERY

RESEARCH OFFICER required to work on an MRC-funded project to establish the identity of neutrophil-derived metabolites of 5 amino-salicylate in order to provide indicators of the progress of inflammatory disease. Candidates should have a first degree in a biochemical or chemical discipline. The project, which is a pilot study, is funded initially for one year but suitably qualified candidates may enrol for a higher degree.

Starting salary up to £10,458 per annum.

Further particulars (quoting Ref. No. S19/2/91) are available from the **Personnel Office, University of Wales College of Medicine, Heath Park, Cardiff, CF4 4XN** (Tel. No. 0222/755944 Ext. 2296) and more detailed information from Dr. M. D. Hallett (Ext. 2748/3). Application in the form of a curriculum vitae with the names and addresses of two referees to the Personnel Officer by January 5th. (2248)A

UNIVERSITY OF ABERDEEN
DEPARTMENT OF GEOLOGY AND PETROLEUM GEOLOGY
OCCIDENTAL POSTDOCTORAL RESEARCH FELLOW IN PETROLEUM GEOLOGY

The appointee will work in collaboration with Dr N H Trewin of the University and the geological team at Occidental Petroleum (Caledonia) Ltd in Aberdeen. The research project is a study of the mineralogy and derivation of late Jurassic and early Cretaceous reservoir sandstones of the Witch Ground Graben, N Sea. Postgraduate experience in sedimentary petrology, sedimentology, diagenesis and log interpretation is preferred. Publication of results is expected. The appointment will be for one year and the salary will be within the range £10,458–£14,703. Candidates should be available to start in March 1990.

Applications by letter with CV and names of 2 referees to the **Personnel Office, University of Aberdeen, Regent Walk, Aberdeen AB9 1FX** by 31 January 1990 quoting reference number JA/079.. (2254)A

QUEEN MARY AND WESTFIELD COLLEGE
(University of London)
School of Mathematical Science (Astronomy Unit)
Research Position in Astronomy

Applications are invited for a Post Doctoral position in Professor Rowan Robinson's IRAS group, currently working on cosmology, starburst and active galaxies, background radiation and star formulation. The post is for an observational astronomer to carry out ground-based follow-up of the IRAS and ROSAT missions. Programming experience and a background in astronomy are essential. Salary will be in the range of £12,738 – £15,177, depending on age and experience. The position is funded by SERC for two years in the first instance, to start as soon as possible.

Please apply to **Personnel Office, Queen Mary and Westfield College, Mile End Road, London E1 4NS.** Applications by CV which should include a publication list, summary of research experience and the names and addresses of two referees. Applications should arrive no later than 2nd January 1990) and quote reference no 89/221. (2274)A

THE AUSTRALIAN NATIONAL UNIVERSITY
Research School of Biological Sciences
MOLECULAR NEUROBIOLOGY GROUP
POSTDOCTORAL FELLOW/ RESEARCH FELLOW

A postdoctoral position is available to study mechanisms of motoneuron pathfinding in insects. The work initially will be on grasshopper, with possible extension to Drosophila. Either a cellular approach, involving ablation or blocking of putative guidance cues followed by embryo culture, or a molecular approach, based on cloning the insect homologues of putative vertebrate guidance molecules, may be pursued depending on the background of the successful applicant. Position available from about May, 1990, for up to three years. An appropriate PhD is required, with three years postdoctoral research experience being desirable for appointment at the Research Fellow level. Enquiries to Dr Eldon Ball, Molecular Neurobiology Group, RSBS, PO Box 475, Canberra City, ACT 2601, Australia. Phone 61-62-49-4496, FAX 61-62-48-9995 or 61-62-49-0758.

Closing date: 15 February 1990. Ref: BS 16.11.1

Salary: Research Fellow: A$32,197-41,841 p.a.; Postdoctoral Fellow Grade 1 (fixed point): A$27,953-31,808 p.a. Applications should be submitted in duplicate to the Registrar, The Australian National University, GPO Box 4, Canberra ACT 2601, Australia, quoting reference number and including curriculum vitae, list of publications and names of at least three referees. The University reserves the right not to make an appointment or to appoint by invitation at any time. Further information is available from the Registrar, or from Appointments (37255), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF.

The University is an equal opportunity employer (W6787)A

POSTDOCTORAL POSITION

available now to study myeloid membrane structure/function. Projects involve granulocyte membrane protein purification and characterization of molecules recognized by myeloid-specific monoclonal antibodies. Send CV and three names or letters of reference to: **Dr. Keith Skubitz, Department of Medicine, Box 325 UMHC, University of Minnesota Medical School, Minneapolis, Minnesota 55455.** Equal Opportunity Employer.

(NW4709)A

THE AUSTRALIAN
NATIONAL UNIVERSIT
JOHN CURTIN SCHOO
OF MEDICAL RESEARC
DIVISION OF
NEUROSCIENCE
POSTDOCTORAL
FELLOW/RESEARCH
FELLOW

A position is available at the leve
Postdoctoral Fellow or Resea
Fellow, to work in collaborat
with Professor Peter Gage on
gating and characteristics of
channels (particularly sodium
calcium) involved in rhyth
firing of cardiac muscle
neurons, and the mode of actio
antiarrhythmic drugs and natur
occurring modulators. Labora
ies are well equipped with el
trophysiological equipment incl
ing patch clamp amplifiers
laboratory computers. Experie
in electrophysiology procedure
desirable.

Enquiries: Professor Peter Ga
(062) 49 2593. Ref. JC 29.11.3

Salary: Research Fellow: A$32,
– A$41,841 pa; Postdoctoral Fell
Grade 1 (fixed point) A$27,953
A$31,808 pa. Appointment: Sen
Research Fellow/Research Fell
up to three years, possibility of
tension to five years; Postdocto
Fellow normally two years, pos
bility of extension to three yea
Applications should be submitt
in duplicate by 2 February 1990
the **Registrar, The Australi
National University, GPO Box
Canberra ACT 2601, Austral**
quoting reference number a
including curriculum vitae, list
publications and names of at lea
three referees. The Universi
reserves the right not to make
appointment or to appoint
invitation at any time. Furth
information is available from t
Registrar, or from Appointmen
(37269), Association of Commo
wealth Universities, 36 Gord
Square, London WC1H 0PF.

*The University is an Equal Opp
tunity Employer.*

(W6805)A

A position is available at th
University of Pennsylvani
for a

Research Assistant Professor in Pediatrics.

The applicant should be a molecul
biologist who will investigate PDG
modulated gene expression. Cand
dates must have thorough knowledg
of molecular biological technique
and protein purification includin
HPLC.

Please send CV and three letters
reference to **Charles Scher, M.D
Children's Hospital of Philadelph
3400 Civic Center Blvd, Philadelphi
PA 19104.**

*The University of Pennsylvania is a
equal opportunity, affirmative actio
employer. Women and minorities ar
encouraged to apply.* (NW4683)A

THE SALK INSTITUTE

Post-Doctoral Position in Analytical Biochemistry

The Clayton Foundation Laboratories for Peptide Biology of the Salk nstitute for Biological Studies is seeking a Post-Doctoral Fellow to erform characterization of new bioactive peptides and proteins sing gas phase sequencing. The ideal candidate will have experi- nce in protein chemistry and enzymology.

Interested individuals may submit a curriculum vitae and a statement of research experience and interests to:

The Salk Institute for Biological Studies
Personnel/Research Chemist
P.O. Box 85800
San Diego, Ca 92138-9216
EEO Employer M/F/V/H

(NW4707)A

ETH ZURICH

Geological Institute

CTURESHIP AND RESEARCH IN TECTONICS

plications are invited for a earch position (potentially ure-track) to complement our earch in structural geology and develop new programs in ional tectonics. A solid back- und in modern structural geol- is expected. The development ield projects and supervision of ctoral and diploma theses in peration with the existing earch groups in petrology, imentology and geophysics is ential. Further information on sting facilities and working con- ions is available on request.

Starting date October 1, 1990, .D. degree required. Appli- nts should submit a detailed umé, publication list, a state- nt of research interests, and mes of three referees by March 1990 to Chairman, Geological stitute ETH, 8092 Zurich, itzerland. (W6795)A

NOTTINGHAM HEALTH AUTHORITY

CITY HOSPITAL

OP GRADE CYTOGENETICIST

esponsible for the provision of e South Trent Cytogenetics ser- ce, the post is based at the City ospital, Nottingham and will form rt of the Nottingham Inter-dis- plinary centre for Medical Gen- ics, comprising Cytogenetics, linical Genetics (NHS and aca- emic), and Molecular Genetics, nd also has input from the Pre- atal Diagnostic Sub-division of e Department of Obstetrics and ynaecology.

Informal enquiries to: Professor R Johnson, ext 46666.

pplication form and job des- iption from the Personnel De- artment, Unit Headquarters, City ospital, Hucknall Road, Notting- am NG5 1PB, Tel: (0602) 627672 24 hour answering service).

Closing date: 22nd December 989. (2252)A

SUNDERLAND HEALTH AUTHORITY

LEUKAEMIA LYMPHOMA RESEARCH LABORATORY

POST DOCTORAL RESEARCH ASSISTANT

Required from January/February 1990 to fill vacancy arising from present incumbent gaining post in U.S.A. Appointment initially for 1 year, renewable subject to performance. This is a spacious, well equipped laboratory, generously funded.

Experience in culture, stimulation and functional analysis of human blood mononuclear cells essential and familarity with ELISA techniques expected. Current research includes study of immune consequences of blood transfusion, immunopathology of Hodgkin's and other lymphomas.

Salary on scale £10,458 – £16,665, according to experience and qualifications. Drive, initiative and enthusiasm are essential and will be rewarded.

For further details/visit to department, Tel. 091 565 6256 ext. 248. (Dr. Kay).

We aim to be an Equal Opportunities Employer. (2283)A

Laboratory Director for a Molecular Biology Resources Facility

Ph.D. or M.S. degree in biochemistry or organic chemistry, preferably with training and several years of experience in analytical biochemistry. The Director supervises the operation of a laboratory that performs amino acid analysis, peptide synthesis, peptide sequencing, and oligonucleotide synthesis for researchers in the biological sciences. Supervise one full-time and two part-time technicians. Send resume and names and addresses of 3 references to **Dr. Raymond W. Beck, Biology Consortium, M-303 Walters Life Sciences Building, University of Tennessee-Knoxville, Knoxville, TN37996.** Application review begins 2/01/90. *UTK is an EEO/AA/Title IX/Section 504 Employer.*

(NW4689)A

UNIVERSITY OF LONDON

INSTITUTE OF PSYCHIATRY

Lectureship in Molecular Cytogenetics

Applications are invited for a post of Lecturer in Molecular Cytogenetics. The successful candidate will be expected to develop research and teaching and to provide a clinical cytogenetics service to the Bethlem and Maudsley Hospitals. Applications are invited from individuals with experience in cytogenetics and/or molecular genetics. Opportunities for training will be provided in Areas in which the candidate is inexperienced, and it is anticipated that part of the lecturer's time will initially be spent at the Genetics Unit at Guy's Hospital.

Salary on the non-clinical lecturer scale.

Applications in the form of a curriculum vitae with the names and addresses of two referees should be submitted to the **Secretary, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF,** from whom further particulars may be obtained. Closing date 12 January, 1990. (2272)A

LINUS PAULING INSTITUTE OF SCIENCE AND MEDICINE

POSTDOCTORAL POSITION IN CHROMATIN

Applications are invited for the above position, available immediately, to study the role of poly(ADP-ribosylation) in chromatin structure, repair and replication. Experience with recombinant DNA techniques, as well as Southern blotting, would be an advantage.

Salary: $25,000 per annum plus fringe benefits and travel expenses.

The Linus Pauling Institute is located in Palo Alto, near Stanford University. Please send your curriculum vitae, publications list and two letters of recommendation to:

Teni Boulikas, Ph.D.,
Head, Laboratory of Molecular Carcinogenesis
Linus Pauling Institute
440 Page Mill Road, Palo Alto, CA 94306

(NW4703)A

POSTDOCTORAL/ RESEARCH ASSOCIATE

Available immediately. Research on species- and tissue-specific enzyme compartmentation. General biochemical background required, experience with protein and rDNA technologies preferred. Salary competitive.

Contact **Dr. James W. Campbell, Dept. Biochem. Cell Biol., Rice Univ., P.O. Box 1892, Houston TX 77251.**

An Equal Opportunity/Affirmative Action Employer. (NW4704)A

UNIVERSITY OF WALES COLLEGE OF MEDICINE

DEPARTMENT OF MEDICINE

INSTITUTE OF NEPHROLOGY (INCORPORATING THE K.R.U.F. INSTITUTE)

RESEARCH OFFICER required from 1 January 1990 for a period of two years. The successful applicant will work on a project to continue current research into the role of lipids in renal disease using glomerular cell culture techniques already established in the Department. Experience in lipid biochemistry would also be helpful.

Salary will be on the scale for University Research and Analogous Staff Grade 1A (£10,458-£16,665 p.a.) starting point dependent on age and experience, up to £12,381 per annum.

Further particulars available from the **Personnel Office, University of Wales College of Medicine, Heath Park, Cardiff, CF4 4XN (Tel. 0222/ 755944 Ext. 2296)** to whom applications in the form of a curriculum vitae with the names and addresses of three referees should be submitted within 3 weeks of the appearance of this advertisement.

(2240)A

MRC CLINICAL RESEARCH CENTRE

WATFORD ROAD
HARROW, HA1 3UJ

Postdoctoral Appointment in Molecular Dermatology

Applications are invited for a Postdoctoral Scientist with experience in recombinant DNA techniques to work on the cloning of novel human desmosomal glycoprotein genes. These cell-surface components play an important role in keratinocyte adhesion and are also of interest as markers of epidermal differentiation.

Antibody and oligonucleotide probes are available for screening cDNA libraries and experience in this field is essential.

The appointment will be for 3 years in the first instance at the appropriate point of the scale for non-clinical university academic staff.

Interested applicants may contact Dr Ian King for further information — telephone (direct line) 01.869.3187.

Application forms are obtainable from the Personnel Dept 01.869.3431. Closing date for completed applications is 2nd January 1990

Reference number: 111/1/3953

The Medical Research Council is an Equal Opportunity Employer. (2290)A

THE UNIVERSITY OF BIRMINGHAM

SCHOOL OF BIOLOGICAL SCIENCES

ELECTRONICS ENGINEER

An electronics engineer is required to take primary responsibility for the design, development and construction of equipment to store physiological data from free-ranging animals. Applicants should have a first degree in electronics (or equivalent) and preferably have experience of low power, subminiature circuitry. The post, funded by NERC, is for three years in the first instance and within the salary range £9,816 to £16,665, according to experience. It may be possible for the successful candidate to register for a higher degree. Proposed starting date 1 February 1990.

Informal enquiries to Professor P. J. Butler (021 414 5470). For application form and further particulars, please contact the **Director of Staffing Services, The University of Birmingham, Edgbaston, Birmingham B15 2TT, telephone 021 414 6483 (24 hour answerphone)** to whom applications (3 copies should be returned by 2 January 1990, quoting reference number 13012).

AN EQUAL OPPORTUNITIES EMPLOYER

(2284)A

Postdoctoral Research Associate

Investigations on multienzyme complex regulated by phosphorylation. Function, regulation, and organization studies with purified complex, subcomplexes, components, and domains. Use monoclonal antibodies and site-directed mutagenesis. Prefer candidates with strong Biochemistry and/or Molecular Biology background. Send resume to **Professor Thomas E. Roche, Willard Hall, Department of Biochemistry, Kansas State University, Manhattan, KS, 66506.** KSU is an equal opportunity affirmative action employer. (NW4691)A

POSTDOCTORAL POSITION

available immediately for x-ray analysis of protein/inhibitor complexes. Experience in crystallography or biophysical chemistry desired. Send curriculum vitae and addresses of three references to **Dr. Brian Edwards, Department of Biochemistry, Wayne State University, 540 E. Canfield Avenue, Detroit, Michigan, 48201.** *Wayne State University is an Equal Opportunity/Affirmative Action Employer.* (NW4696)A

POSTDOCTORAL/ASSOCIATE RESEARCH SCIENTIST

positions available in the Department of Pharmacology at Yale University School of Medicine to study: (1) mechanisms of action of drugs on hypoxic tumour cells with emphasis on the reductive activation of bioreductive alkylating agents, and (2) mechanism of drug induced differentiation of neoplastic cells. Ph.D. required and experience in biochemistry and/or pharmacology desired. Deadline for receipt of applications is January 31, 1990. Send curriculum vitae and three letters of reference to: **Dr. Alan C. Sartorelli, Director, Comprehensive Cancer Center, Yale University School of Medicine, 333 Cedar Street, New Haven, Connecticut 06510.**

Yale University is an Equal Opportunity/Affirmative Action Employer. The Department of Pharmacology encourages applications from Women and Minority Group members. (NW4622)A

UNIVERSITY OF NEWCASTLE UPON TYNE

CLINICAL SENIOR LECTURESHIP AND CLINICAL LECTURESHIP IN THE DEPARTMENT OF CLINICAL BIOCHEMISTRY AND METABOLIC MEDICINE

As part of an expansion and further commitment to research activities applications are invited for two new academic appointments in the Department of Clinical Biochemistry and Metabolic Medicine.

CLINICAL SENIOR LECTURER IN CLINICAL BIOCHEMISTRY

The successful candidate will have proven abilities in research and will want to develop these further. It is expected that he or she will have a higher degree. While suitable applicants in all areas will be considered seriously, those with a background in molecular endocrinology and/or immunology would be particularly welcome. Duties will include teaching at undergraduate and postgraduate levels and participation in the clinical and administrative activities of the Department.

CLINICAL LECTURER IN CLINICAL BIOCHEMISTRY

The successful candidate will want to follow a career in clinical research. Applicants who wish to interact productively with a new Immunology Group being formed in the area of immunogenicity would be particularly welcome. Duties will include teaching at undergraduate and postgraduate levels and participation in the clinical and administrative activities of the Department.

The first appointment will be made at a suitable point on the Clinical Senior Lecturer's salary scale (£29,700-£38,340 p.a. if Honorary Consultant contract held and £23,570-£33,260 p.a. without Honorary Consultant contract). The second appointment will be made at a suitable point on the Clinical Lecturer's salary scale (£14,545-£25,500 p.a.).

Further particulars may be obtained from the **Senior Assistant Registrar (Establishments), The University, 6 Kensington Terrace, Newcastle upon Tyne, NE1 7RU** with whom applications (three copies) together with the names and addresses of three referees should be lodged by the 2nd January 1990. (2277)A

RESEARCH ASSOCIATE OR POST-DOCTORAL FELLOW

Biochemist/Biologist wanted to investigate the molecular genetics of signal transduction in eukaryotes. Project involves identification and characterization of genes whose products are important in cell surface receptor mediated gene induction. Salary: $25,000-$32,000 commensurate with experience and qualifications. Send CV to: **Dr. Robert P. Dottin, Hunter College, CUNY, Center for Study of Gene Structure and Function, 695 Park Avenue, New York, NY 10021.**

(NW4695)A

THE AUSTRALIAN NATIONAL UNIVERSIT

John Curtin School of
Medical Research

Division of Clinical Science

POSTDOCTORAL FELLOV
RESEARCH FELLOW/
SENIOR RESEARCH FELL

An experienced immunolog
cell biologist is required to j
the Developmental Haematol
Group, to work in collaborati
with Dr. A Hapel. Current int
ests of the group include b
marrow differentiation and
role of cytokines and cell surf
receptors in hematopoiesis. T
appointee will be required to de
lop an area of retrovirology rela
to hematolymphoid developme
In particular the group is interes
in the effect of HIV gene expr
sion on cell function. Essen
experience includes gene and ret
virus cell transfer techniques, cl
ing and engineering retroviru
and a full capacity to do functio
analysis of bone marrow and ly
phoid cells.

Enquiries may be directed
Dr. A.J. Hapel, Head of Gro
(062) 49 2381, to Professor W
Doe (062) 49 2392, or to
School Secretary.

Ref. JC 29.11.1

(This position was previou
advertised as Research Fell
Ref. JC 26.10.1, with a clos
date of 8.12.89).

Salary: Senior Research Fell
A$44,397 – A$52,675 pa; Resea
Fellow: A$32,197 – A$41,841
Postdoctoral Fellow Grade
(fixed point) A$27,953 — A$31,
pa. Appointment: Senior Resea
Fellow/Research Fellow up
three years, possibility of ext
sion to five years; Postdocto
Fellow normally two years, po
bility of extension to three yea
Applications should be submit
in duplicate by 25 January 199
the Registrar, The Austral
National University, GPO Box
Canberra ACT 2601, Austral
quoting reference number a
including curriculum vitae, list
publications and names of at le
three referees. The Univers
reserves the right not to make
appointment or to appoint
invitation at any time. Furt
information is available from
Registrar, or from Appointme
(37268), Association of Comm
wealth Universities, 36 Gord
Square, London WC1H 0PF.

The University is an Eq
Opportunity Employer.

(W6804)A

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nature

Still first with the bes
in science

FELLOWSHIPS

The Royal Society

RESEARCH FELLOWSHIPS VACANT
from 1 October 1990

The following 40 appointments have recently been advertised to be taken up on 1 October 1990, viz:

University Research Fellowships (28) in any scientific subject for candidates preferably between 26 and 33

Research Fellowships (4) in the medical, biological and chemical sciences for candidates preferably between 33 and 40

The Royal Society-BOC Limited Research Fellowship (1) for a subject of research in a field of direct interest to BOC for applicants preferably under 33

Rutherford Scholarships (2) for candidates under 26 to do a Ph.D in a part of the Commonwealth different from that in which the candidate graduated

Royal Society Leverhulme Trust Senior Research Fellowships (5) for candidates between 35 and 55 with substantive posts in British universities to do full-time research for one year

Details of all the vacancies appeared in **Nature** on 16 November 1989, (classified, p.17), giving information on eligibility and conditions, including permitted nationality of applicants and places of tenure of the appointments.

Apply by letter for application forms and further details (**stating for which appointment**):
Mrs U. M. A. Tokle, The Royal Society, 6 Carlton House Terrace, London, SW1Y 5AG.

(2264)E

Closing Date: 16 February 1990
No application forms will be issued after 2 February 1990 and no applications arriving after 4 p.m . on 16 February will be considered.

APPTS. VACANT
continued

UNIVERSITY OF BRISTOL

SCHOOL OF CHEMISTRY

LECTURESHIP IN BIO-ORGANIC CHEMISTRY

Applicants are invited under the New Academic Appointments Scheme which is intended to create career opportunities for younger academic staff. Salary will be assessed on the Grade A scale (£10,458–£15,372).

Applications, in the form of a detailed curriculum vitae with an explanatory letter giving names and telephone numbers of three referees, should be sent to **the Registrar, Senate House, Bristol, BS8, 1TH, from whom further details may be obtained. Please quote reference CYF 3.**

Closing date 31 January 1990.

An equal opportunities employer. (2282)A

FOULKES FOUNDATION FELLOWSHIPS 1990

The Foulkes Foundation provides financial support for recently qualified science graduates with research experience who want to study medicine, and for medical graduates who want to take a science degree. In both cases graduates must intend to do medical research after qualification.

Application forms and further information available from the Registrar, Foulkes Foundation Fellowship, 37 Ringwood Avenue, London N2 9NT, enclosing S.A.E.

Closing date — 15th March, 1990. (2278)E

Harvard University

announces Bullard Fellowships in Forest Resources with stipends available for advanced study and research. Fellowships are open to individuals in academic careers, public service, or private forestry with stipends up to $25,000. Further information may be obtained from: **Committee on the Charles Bullard Fund for Forest Research, Harvard University, Harvard Forest, Petersham, Massachusetts 01366.** Deadline for applications: February 1, 1990. (NW4697)E

EMBO

European Molecular Biology Organization

LONG TERM FELLOWSHIPS IN MOLECULAR BIOLOGY SPRING 1990 AWARDS

Next deadline: 15th February 1990

EMBO long term post-doctoral fellowships are awarded to promote the development of molecular and allied research in Europe and Israel. To be eligible a candidate must hold a doctorate degree and the exchange must involve a laboratory in Western Europe or Israel. EMBO fellowships are not, however, awarded for exchanges between laboratories within any one country. Long term fellowships are usually awarded initially for one year, but with the possibility of renewal for a second year. In cases of exceptional scientific merit, however, the selection committee can award a 2-year fellowship. The fellowship comprises a return travel allowance for the fellow and any dependents and a stipend and dependents' allowance.

Since the selection procedure may include an interview, candidates are requested to respect the deadline for complete applications which is 15 February 1990. Successful candidates will be notified of their awards immediately after the meeting of the selection committee, which is on 23 April 1990.

Application forms and further details may be obtained from **Dr. J. Tooze, Executive Secretary, European Molecular Biology Organization, Postfach 1022.40, 69 Heidelberg 1, F.R. Germany.** Inquiries should be accompanied by a self-addressed adhesive label. (W6801)E

THE ARTHRITIS & RHEUMATISM COUNCIL

ARC

ARTHRITIS & RHEUMATISM COUNCIL FOR RESEARCH

SENIOR A R C FELLOWSHIPS

The purpose of these fellowships is to provide support for outstanding medical or basic science graduates committed to a research career in any aspect of the rheumatic diseases. Candidates should have proven research ability in a relevant discipline.

These fellowships will normally be for 5 years, with possible renewal, subject to satisfactory review. They are tenable in universities and research institutions in the UK only.

Salary would be according to age and experience on appropriate salary scales. Funds would also be available for a technician, laboratory expenses and essential equipment not normally provided for by departmental funds.

Applications should include evidence of appropriate support and facilities from the host department.

Application forms and further information are available from the

Research & Education Secretary,
The Arthritis & Rheumatism Council,
41 Eagle Street,
London WC1R 4AR.

Completed applications should be received by Thursday, 1 March 1990. (2276)E

LUCILLE P. MARKEY VISITING FELLOWSHIPS

Nominations are invited for postdoctoral fellowships under the above programme. Four awards will be made to outstanding candidates wishing to further their careers in biomedical science at an institution in the U.S.A. Candidates *must* be nominated by an institution in the U.K. and individual applications will not be considered. The awards are for a two-year period, with a stipend of $30,000 in the first year and $33,000 in the second, plus a $750 travel allowance each year. Funding for a third year may be possible by reapplication. The Markey postdoctoral fellowships are for study of fundamental biological phenomena (rather than specific diseases) in fields such as molecular biology, developmental biology, neurobiology, immunology, genetics and virology.

Further information and application forms can be obtained from:

Dr. G. Stark
Imperial Cancer Research Fund
P.O. Box 123
Lincoln's Inn Fields
London WC2A 3PX

Closing date for completed applications is 1st April 1990.

Applicants will be notified by early June and awards can be taken up soon thereafter. (2181)F

Mental Health Foundation

Career Development Fellowships for Research into Mental Disorders

Applications are invited from medical and scientific graduates for a Fellowship for research training in the field of mental disorder.

These awards which are tenable for up to 3 years, are intended for clinicians with a training in psychiatry or intending to train in psychiatry, or postdoctoral scientists who wish to extend their research experience. The research proposals should be relevant to mental health or related basic science and the applicant should intend to continue research in this field. These Fellowships may be held in appropriate departments or clinical centres in Great Britain. The Trustees are prepared to consider a request that part of the Fellowship be spent in a suitable centre, or centres, abroad in cases where the necessary research training cannot all be obtained in Great Britain. Candidates are welcome to discuss their plans with the Honorary Secretary of the Research Committee.

Candidates should not normally be over 32 years of age for science graduates and 34 years for medical graduates. However, consideration will be given to those over this age where their career has been affected either by a late start or by interruption for personal/family reasons. Applications should be made jointly with the Head of the Department in which the candidate proposes to work. Salaries will be in the range of University Lecturer or NHS Registrar scales (whichever is appropriate) according to age and experience.

Application forms can be obtained by writing to
The Secretary of the Research Committee,
The Mental Health Foundation, 8 Hallam Street, London W1N 6DH.

Applications must be returned by March 1st.
(Late applications will not be considered). (2242)E

MOORFIELDS EYE HOSPITAL
HOCK FELLOWSHIP IN OPHTHALMOLOGY

Thanks to the generosity of the original donors, a new fellowship has been established, tenable for three years at Moorfields Eye Hospital and/or at the Institute of Ophthalmology.

The research undertaken by the successful fellow should contribute to knowledge of diseases of the retina and vitreous. Renumeration will depend upon experience, but it will be up to Lecturer grade. The proposed research will be supervised by a Head of Department at the Institute of Ophthalmology. Candidates should ensure that the details of their project have been approved by their supervisor before submitting the application for the Fellowship.

Applications, together with C.V.s and the names of two referees should be received by 18th January 1990.

Medical Staffing Officer, Moorfields Eye Hospital, City Road, LONDON EC1V 2PD. (2246)E

1990 BEIT MEMORIAL FELLOWSHIPS FOR MEDICAL RESEARCH

Applications are invited by the Trustees, for up to six Beit Memorial Fellowships for Medical Research. The aim of the Fellowships is to promote the advancement by research of medicine and the allied sciences in their relation to medicine. One or two of the Fellowships will be awarded to a candidate whose research proposals are judged to reach the appropriate standard and to bring benefit to the inhabitants of Zimbabwe, Malawi or Zambia, but separate application is not required. The salary will be in the range of a Lecturer or Clinical Lecturer, according to age, previous experience and qualifications of the applicant (maximum starting salary in the range £11,088-£15,372 pa plus London Allowance of £1,650 where appropriate). There will be the usual University superannuation contributions and benefits. Some reseach expenses may be granted on application. The usual tenure of a Fellowship is three years.

Eligibility — in conformity with the conditions under which the Fellowships were first established, every Fellow shall be a man or woman of any nationality whatsoever who at the date of election shall have taken a degree in any faculty in any University approved by the Trustees, in the U.K. or an any Country which is or has been since 1910 a Dominion, Protectorate or Mandated Territory of the Crown. Elections to the Fellowships are rarely made above the age of 35 years. Candidates must submit written evidence that they will be given accommodation in the department where they propose to work which must be in Great Britian or Ireland and their research proposal must be agreed with and confirmed in writing by the Head of the Department.

Forms of application and information may be obtained by writing to **Miss D Billington, Admin. Secretary, Beit Memorial Fellowships for Medical Research, The School of Pharmacy, 29-39 Brunswick Square, London WC1N 1AX. Completed application forms must be received by 15 March 1990.** (2245)E

HOWARD HUGHES MEDICAL INSTITUTE

POSTDOCTORAL RESEARCH FELLOWSHIPS FOR PHYSICIANS

1990 Competition

Fellowships are for full-time training in fundamental research on basic biological processes and disease mechanisms.

Fellowship Terms

3 years of support
$35,000-$50,000 stipend
$15,000 research allowance
$12,000 institutional allowance

Eligibility

M.D., M.D./Ph.D., D.O.
At the start of the fellowship
at least two years of clinical training and
no more than three years of research training
No citizenship requirements

Schedule

Application deadline: **March 1, 1990**
Awards announced: **October 1990**
Fellowships start: **January 1991 — July 1992**

For Program Announcements and Applications

Howard Hughes Medical Institute
Office of Grants and Special Programs
6701 Rockledge Drive
Bethesda, MD 20817
United States of America
Telephone (301) 571-0324

The Howard Hughes Medical Institute, an Equal Opportunity Employer, welcomes applications from all qualified candidates and encourages women and members of minority groups to apply.

(NW4151)E

UNIVERSITY OF WARWICK
Department of Biological Sciences
POSTDOCTORAL FELLOW

The above post is available immediately for suitably qualified candidates with expertise in molecular biology. The successful applicant will join a small team working on biochemical methane oxidation whose current goals are centered on the mechanism of action and site-directed mutagenesis of the hydroxylase component of the enzyme system methane monooxygenase. The fellowship, funded by the Gas Research Institute of Chicago, is available for two and a half years.

Salary will be on the Research IA scale: £10,458 to £16,665 p.a. Further details about the project may be obtained from Dr. J.C. Murrell (0203 523553) or Professor H. Dalton (0203 523552) (direct lines). Application forms from the **Executive Officer, Department of Biological Sciences, University of Warwick, Coventry, CV4 7AL,** quoting Ref. No. GRI/BS332/14. *Closing date: 7th January 1990.* (2244)E

UNIVERSITY OF BRISTOL
Department of Pathology
Tumour Invasion and Metastasis

A post-doctoral fellowship funded by the Cancer Research Campaign is available from January 1990 or as soon as possible thereafter for 3 years to work on a project concerning the role of cysteine proteinases and their inhibitors in tumour invasion and metastasis in colorectal carcinogenesis. The person will join an existing team currently working on the cellular and molecular biology of human colorectal carcinogenesis. Experience in biochemistry, cell and/or molecular biology, tissue culture advantageous, although training can be given in these areas.

Salary within the range £10,458–£16,665 (Research 1A) according to age, qualifications and experience.

For further particulars telephone Bristol (0272) 303136 (ansaphone after 5.00 pm) or write to the **Personnel Office, University of Bristol, Senate House, Bristol BS8 1TH.** Further information may be obtained from Dr Rose Maciewicz (0934–852661 ext. 282) or Dr Chris Paraskeva (0272—303454). (2280)E

UNIVERSITY OF OXFORD
DEPARTMENT OF EXPERIMENTAL PSYCHOLOGY
RESEARCH ASSISTANTSHIP(S) IN THE NEUROPHYSIOLOGY OF MEMORY

Applications are invited for postdoctoral or graduate positions to analyse the activity of single neurons in the hippocampus and visual association cortex of the primate. Applications will be particularly welcome from scientists with interests in neurophysiology, mechanisms of learning and memory, and/or information processing in the cerebral cortex. The salaries are on the RS1A scale, £10,458-£16,665, or the RS1B scale £9,816-£14,169. Applications including the names of two referees, or enquiries, to **Dr. E.T. Rolls, University of Oxford, Department of Experimental Psychology, South Parks Road, Oxford OX1 3UD, England.** (2259)P

University of Glasgow
Department of Bacteriology and Immunology

Research Assistantship (Grade 1B) available immediately to join a research group working on different aspects of mucosal immunity. This 3 year project grant, funded by Action Research for the Crippled Child, will develop in vitro models to study the influence of the immune system on intestinal epithelial cells. Candidates should have a good honours degree in cell biology, immunology or a related subject and will be expected to register for a higher degree.

Salary will be £9816 on Research Assistant 1B scale.

Further details from and applications to **Dr. Allan Mowat, Department of Bacteriology and Immunology, Western Infirmary, Glasgow. G11 6NT (041 339 8822 ext. 4498).** (2249)P

POSTDOCTORAL FELLOWSHIPS in the Cognitive Neuroscience of Human Language for Ph.D.'s and M.D.s available immediately at Johns Hopkins' Mind/Brain Institute. The Cognitive Neuroscience program at Johns Hopkins is a multidisciplinary collaboration among researchers in Cognitive Science, Neurology, Neuroradiology, and other departments. Ongoing research emphasizes the functional and neuranatomic organization of language, using the methods of cognitive neuropsychology, behavioral neurology, and direct electrical stimulation and recording from the human cortex, with ^{15}O PET studies planned. The focus of the fellowships will be on cross-disciplinary exposure to research issues and methods; each fellow will have two advisors, from the cognitive sciences and the neurosciences. Positions available for Ph.D.s in Cognitive Psychology, Cognitive Science, Linguistics, Neurophysiology, or Computational Neurobiology, or for M.D.s who have an interest in Behavioral (Cognitive), Neurology. Send application with vita plus 3 letters of recommendation by March 15, 1990 to **Guy M. McKhann, M.D, or Alfonso Caramazza, Ph.D., Co-Directors, Program in Cognitive Neuroscience, c/o Mind/Brain Institute, 114 Jenkins Hall, Johns Hopkins University, 3400 N. Charles St., Baltimore, MD 21218.** *The Johns Hopkins University is an equal opportunity employer.* (NW4671)E

STUDENTSHIPS

ASTON UNIVERSITY
PHARMACEUTICAL SCIENCES INSTITUTE
RESEARCH STUDENTSHIP

available immediately in behavioural pharmacology to work on the role of 5-HT in tic disorders.

Candidates should already have a first or upper second class honours degree in a biological subject and expect to register for a PhD, although candidates for MPhil by research (1 year) will be considered.

Details: **Dr S L Handley 021-359 3611 ext 4182.** (2270)F

OXFORD CENTRE FOR MOLECULAR SCIENCES
STUDENTSHIPS

The Oxford Centre for Molecular Sciences has a number of SERC, MRC and CASE studentship awards available from October 1990. The core research programme of the Centre is based on the following four topics:—

Protein structure and folding (Dr C M Dobson).

Protein specificity and catalysis (Dr L N Johnson).

Protein-protein interactions (Dr I D Campbell).

Protein-small molecule interactions (Professor J E Baldwin).

The OCMS is an interdisciplinary centre with excellent facilities dedicated to the understanding of the structure, function and reactivity of proteins. The Centre's members are drawn from 9 Oxford Science Departments. Successful applicants will have the opportunity to delay their decision on their proposed area or research until they have explored more fully the work of the OCMS.

The Centre has strong links with industry through collaborative projects and the OCMS Industrial Affiliates club.

If you are interested in finding out more about working for a D. Phil in this exciting environment, send a CV with the names of two referees to:

The Administrator, OCMS, Dyson Perrins Laboratory, South Parks Road, Oxford. OX1 3QY.

Closing date for applications: 26 January 1990. (2269)F

Ph.D. Studentships

A number of PhD Studentships will be awarded in 1990 in the Fund's Laboratories in Central London (Lincoln's Inn Fields and Extra Mural Units), at Clare Hall, N. London, Oxford and Edinburgh for full-time studies leading to higher degrees in research fields relevant to cancer.

The awards will be tenable for three years from 1st October 1990 with a taxable grant £9,736 (Inner London), £8,912 (Clare Hall, Outer London), £8,088 (Oxford), and £7,269 (Edinburgh), for the first year and with annual incremental increases thereafter. In some cases additional allowances are payable.

Early applications are invited from candidates, not more than 25 years of age on October 1st 1990, who have been resident in the UK since October 1987 (although non-residents are not excluded from consideration) and who hope to obtain a First or Upper Second Class Honours Degree. Listed below are the Heads of Laboratories with available projects.

1 **Dr J Armstrong**: Ras-Like Proteins in Fission Yeast Membrane Traffic.

2 **Dr D Bentley**: Regulation of Transcription in Differentiation

3 **Dr R Bicknell**: Molecular Mechanisms of Angiogenesis.

4 **Dr D A Cantrell**: Signal Transduction Mechanisms in T Lymphocytes.

5 **Dr M M Coombs**: A Problem of General Relevance in Chemical Carcinogenesis.

6 **Dr J Downward**: Structural and Functional Studies of a Protein that Controls the RAS Oncoproteins.

7 **Dr M Fried**: The Molecular Mechanisms Involved in Mammalian Gene Expression.

8 **Dr A M Frischauf**: Mutated Genes in Hereditary Diseases.

9 **Dr S Goodbourn**: Gene Expression in Embryonal Carcinoma Cells.

10 **Dr P Goodfellow**: Molecular Genetics of Sex Determination and Genome Manipulation.

11 **Prof. A Harris & Dr I Hickson**: Molecular Cloning of a Human DNA Repair Gene.

12 **Dr I Hart**: Molecular Mechanisms of Melanoma Metastasis.

13 **Dr D Ish-Horowicz**: Analysing Transcriptional Control Elements Regulating Striped Expression of the Hairy Segmentation Gene in Drosphila.

14 **Dr N Jones**: Transcriptional Regulation by the Adenovirus EIA Gene.

15 **Dr P Karran**: Regulation of the Expression of a Human DNA Repair Enzyme.

16 **Dr H Land**: Function and Interaction of Co-operating Oncogenes.

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18 **Dr M Meuth**: The effect of DNA Structure on the Pattern of Mutation in Mammalian Cells.

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23 **Dr M Sternberg**: Prediction of Protein Three-Dimensional Structure from Amino Acid Sequence using Computer Graphics – Application to Transmembrane Helices.

24 **Dr J Taylor**: Analysis of Molecular Events in Breast Cancer.

25 **Dr R Treisman**: Regulation of Gene Expression by Growth Factors.

26 **Dr J Trowsdale**: Organisation and Regulation of Human MHC Gene Expression.

27 **Dr G Warren**: The Mechanism of Receptor-Mediated Endocytosis.

28 **Dr S M Watt & Dr B D Young**: Functional Analysis of Stem Cell Proteins.

29 **Dr S C West**: The Molecular Biology of Genetic Exchange.

30 **Dr J G Williams**: The Regulation of Cell-Type – Specific Gene Expression in Dictyostelium.

31 **Dr R Wolf**: Post-Transcriptional Regulation of Cytochrome P450 Expression.

32 **Prof. N A Wright & Dr R A Goodlad**: Luminal Nutrients and Intestinal Cell Division.

For further details and application form, please write or telephone:

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Tel: 01-242 0200
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All three departments have a wide range of current research interests and have excellent laboratory facilities.

Candidates should normally be not more than 25 years of age on 1st October 1990 and have been ordinarily resident in the UK for a minimum period of 3 years, and either possess, or hope to obtain, a First or Upper Second Class Honours Degree in an appropriate subject.

Further information can be obtained from **The Registrar, The Institute of Child Health, 30 Guildford Street, London WC1N 1EH. Telephone 01-242 9789 Ext: 2679.**

Applications in the form of a Curriculum Vitae and the names of two academic references should be submitted as soon as possible. (2273)F

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Mental Health Foundation

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The Research Committee of the Foundation exists to foster and support research in all fields bearing on mental health, mental illness and disorders of mental development.

The Committee meets twice a year to award **RESEARCH GRANTS TO PROVIDE SCIENTIFIC ASSISTANCE, EXPENSES or EQUIPMENT** for research workers.

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September 1st for meeting in November.

For Grant application forms and further details apply in writing to:
The Honorary Secretary, The Research Committee,
Mental Health Foundation,
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Requests for further information, stating the specific field of interest, should be sent to the **Medical Faculty Office, Medical and Biological Sciences Building, University of Southampton, Southampton, SO9 3TU.**

Closing date: 23 December 1989.

Please quote reference number: 165/JAD/jm. (2251)H

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THE LOUIS JEANTET FOUNDATION FOR MEDICINE

NOMINATIONS FOR THE 1991 AWARDS

Nominations are being sought for the awards of the Louis Jeantet Foundation for Medicine for 1991. One to three awards will be made. They will amount to 1.5 million Swiss Francs (approximately US$1,000,000) in 1991. These awards will provide substantial funds for the support of biomedical research projects (fundamental or clinical) of the highest quality. Candidacies in clinical research are strongly encouraged.

Candidates (either individuals or research groups) must be nominated by scientists, physicians or institutions having detailed knowledge of the candidates' research. The awards are not intended to honour past accomplishments but to help and encourage the laureates' continued research activity. Candidates shortlisted for the final selection will therefore be asked to provide a research project to which the financial support of the award could give decisive impetus.

The previous laureates of the Louis Jeantet Award have been Luc Montagnier (France), Michael Berridge (UK) and Désiré Collen (Belgium) in 1986, Sidney Brenner (UK), Walter Gehring (Switzerland) and Dominique Stehelin (France) in 1987, Bert Sakmann (West Germany), John J. Skehel (UK) and Rolf Zinkernagel (Switzerland) in 1988, Roberto J. Poljak (France), Walter Schaffner (Switzerland) and Greg P. Winter (UK) in 1989. The name(s) of the laureate(s) of the 1990 Louis Jeantet Award will be announced on January 16, 1990.

The following general points should be noted:

1. Applications received directly from candidates will not be considered by the Science Committee.
2. The awards will be limited to researchers working in one of the following countries:
   Austria, Belgium, Denmark, Finland, France, Greece, Ireland, Italy, Norway, Portugal, Spain, Sweden, Switzerland, The Federal Republic of Germany, The Netherlands, The United Kingdom or Yugoslavia,
   although they need not be themselves nationals of any of those countries.
3. All the proposals must be submitted, confidentially, on the official forms only. These are obtainable from:

   The Secretary of the Science Committee
   The Louis Jeantet Foundation for Medicine
   P.O. Box 277
   CH – 1211 GENEVA 17 Malagnou
   Switzerland

   Further information will be sent with the nomination form.
4. The deadline for applications is February 15, 1990.

The name(s) of the laureate(s) of the 1991 Louis Jeantet Award will be announced in January 1991.

(W6797)N

Royal Postgraduate Medical School

(University of London)

Immunology Update for Clinicians

A revision course for clinicians and scientists

16–25th May 1990

The course is divided into two parts: part A (days 1–3) provides an introduction to fundamental immunology, part B (days 4–8) is designed to bring participants up to date with the current concepts and progress in this field and to relate these concepts to clinical problems. Students may attend either part, but the course is designed as a continuum.

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Course Organisers: Dr A Zumla, Dr A N Warrens, Prof J Batchelor, Dr R I Lechler and Dr M A Ritter.

Course fee (inc. catering):

Part A:	**£195**	3 days
Part B:	**£325**	5 days
Parts A & B:	**£480**	8 days

Further details from:

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(2253)C

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Co-directors: M. R. Hughes and B. W. O'Malley.

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For further information contact: **Bonnie Cuthbert, Department of Cell Biology, Baylor College of Medicine (713)-798-6200.**

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MARINE BIOLOGICAL LABORATORY

1990 Summer Courses

BIOLOGY OF PARASITISM (June 10-August 10) Daily lectures and laboratory for 16 advanced students on molecular parasitology covering the immunology, molecular biology and biochemistry of the interactions between parasites and their host. Co-Directors: John E. Donelson, University of Iowa School of Medicine and Carole Long, Hahnemann Medical College.

EMBRYOLOGY: CELL DIFFERENTIATION AND GENE EXPRESSION IN EARLY DEVELOPMENT (June 21-July 30) An advanced level course intended for graduate students, postdoctoral fellows, and other research scientists who wish a current treatment of major issues in the experimental analysis of developmental biology of the embryo, and the diverse intellectual approaches now useful in this field. Limited to 24 students. Director: Eric H. Davidson, California Institute of Technology. Assistant Director: J. Richard Whittaker, Marine Biological Laboratory.

MOLECULAR PROBES IN MARINE ECOLOGY: CONCEPTS, TECHNIQUES AND APPLICATIONS (June 17-July 28) An intensive course for graduate students, postdoctoral fellows, and established investigators who wish to learn the techniques of molecular biology and their application to physiological and ecological investigations. Lectures and discussions will accompany the laboratories and will focus on the technologies being learned and applications to specific problems in marine ecology. Limited to 24 students. Director: J. Woodland Hastings, Harvard University.

MICROBIOLOGY: MOLECULAR ASPECTS OF CELLULAR DIVERSITY (June 10-July 26) An intensive course intended for graduate or postdoctoral students as well as established investigators who desire to become competent in microbiological techniques for working with a broad range of microbes. Limited to 20 students. Co-Directors: Martin Dworkin, University of Minnesota and John Breznak, Michigan State University.

NEURAL SYSTEMS AND BEHAVIOR (June 10-August 1) Intended for graduate and postdoctoral students as well as established investigators. The central theme of this course is the neural bases and ontogeny of behaviors. Twenty students participate in intensive laboratories/lectures which combine state-of-the-art neurobiological techniques with behavioral and developmental analyses. Co-Directors: Ronald Calabrese, Emory University and Martha Constantine-Paton, Yale University.

NEUROBIOLOGY (June 10-August 11) For 12 pre- and postdoctoral fellows who intend to work in cellular and molecular neurobiology. Postdoctorals and students at an advanced stage of their graduate training are particularly encouraged to apply. The themes will be the functions of neural cells, the molecules involved in these functions, and the organization of molecular components required to generate cellular activity. Co-Directors: Leonard Kaczmarek, Yale University; Irwin Levitan, Brandeis University; Christopher Miller, Brandeis University.

PHYSIOLOGY: CELL AND MOLECULAR BIOLOGY (June 10-July 21) For a class of 36 graduate students, postdoctoral fellows and established investigators who desire to learn the methods and research strategies used to study physiology at the cellular and molecular levels. Recent college graduates on their way to graduate school and physicians reentering laboratory research are also encouraged to apply. Interested students may apply for 2 weeks of postcourse research starting July 23. Director: Thomas D. Pollard, Johns Hopkins University.

Application Deadline: March 1, 1990
Partial financial aid is available to qualified applicants.
Contact: Admissions Coordinator, Office of Sponsored Programs,
Marine Biological Laboratory, Woods Hole, MA 02543
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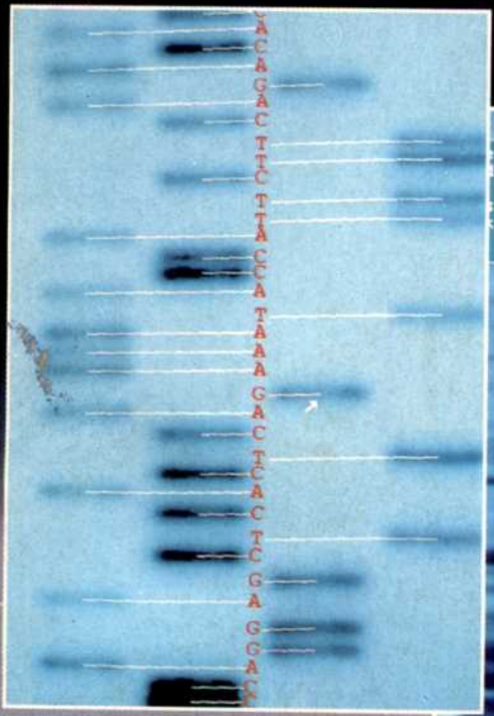

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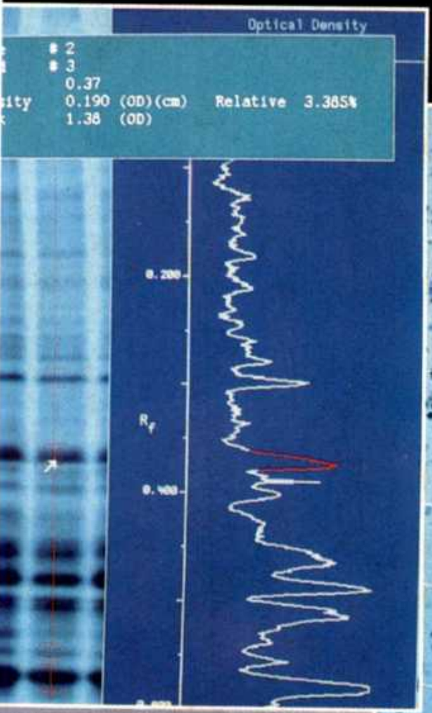

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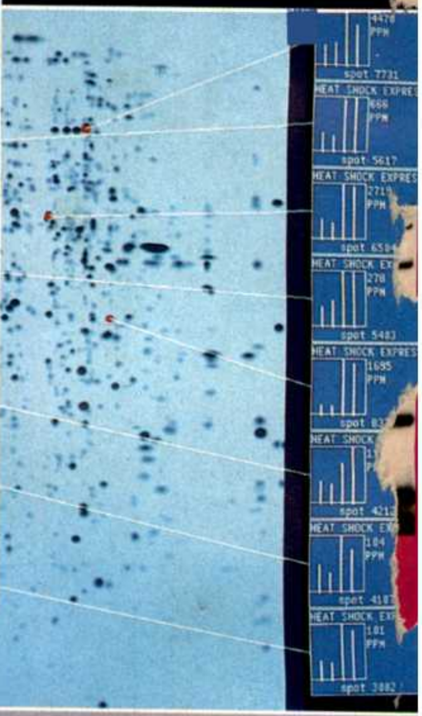

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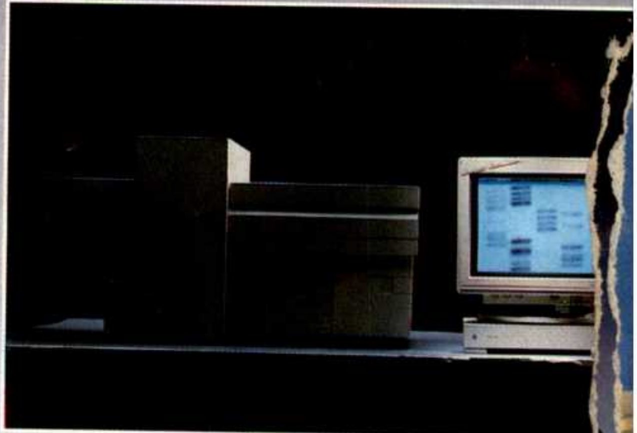

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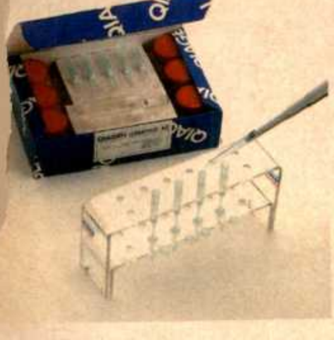

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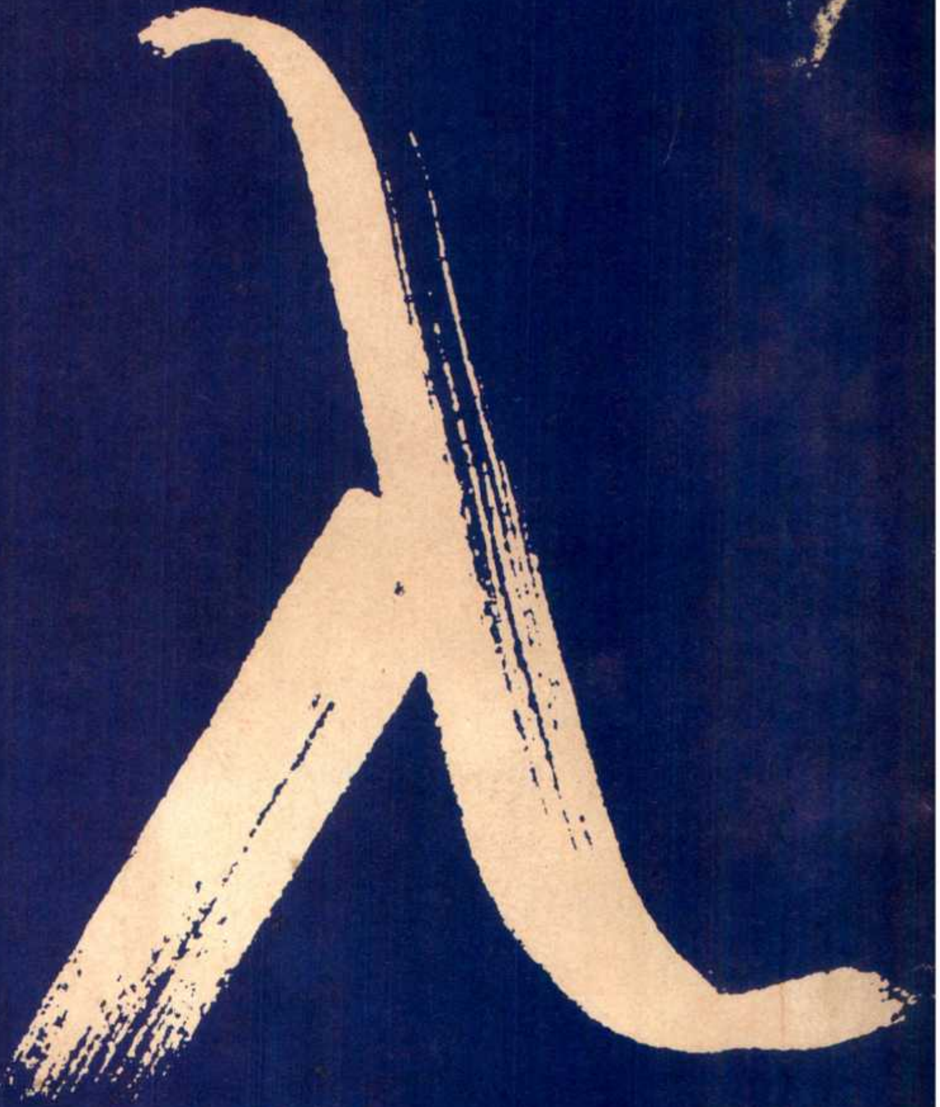

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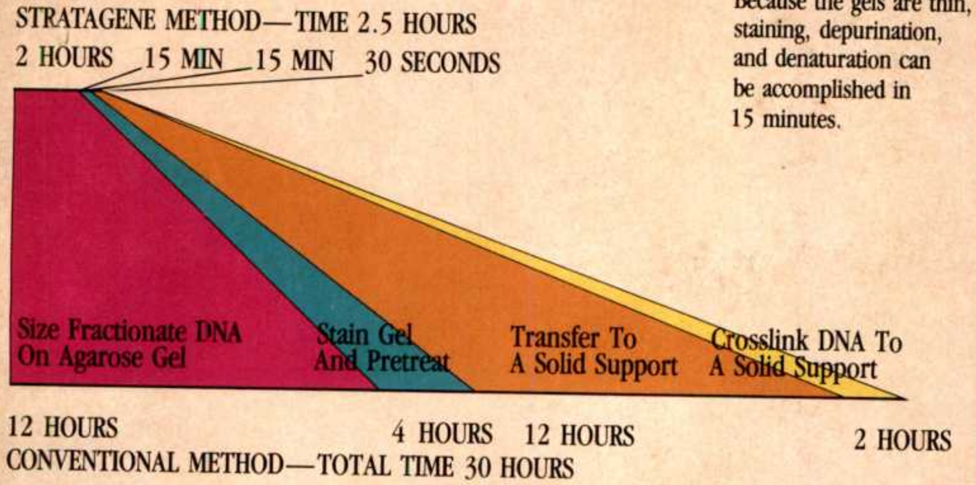

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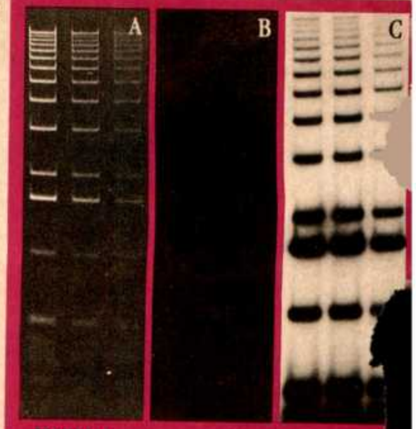

FIGURE 1:

Figure Legend: Fractionation of end labeled DNA markers on 3mm thick 0.8% agarose by the VAGE apparatus and transfer to Duralon—UV™ membranes using the PosiBlot pressure blotter.

- A. Ethidium stained gel showing high resolution.
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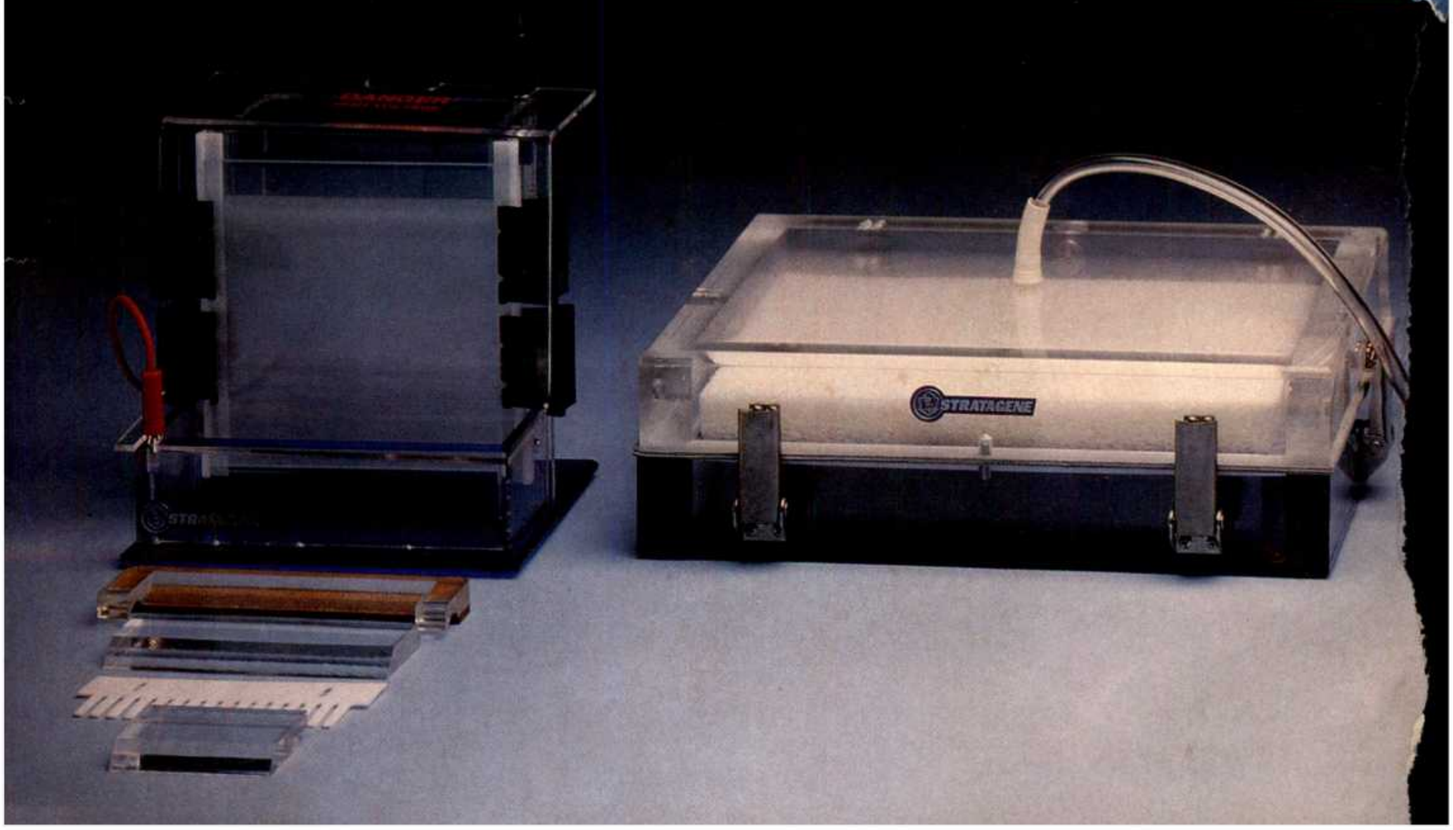

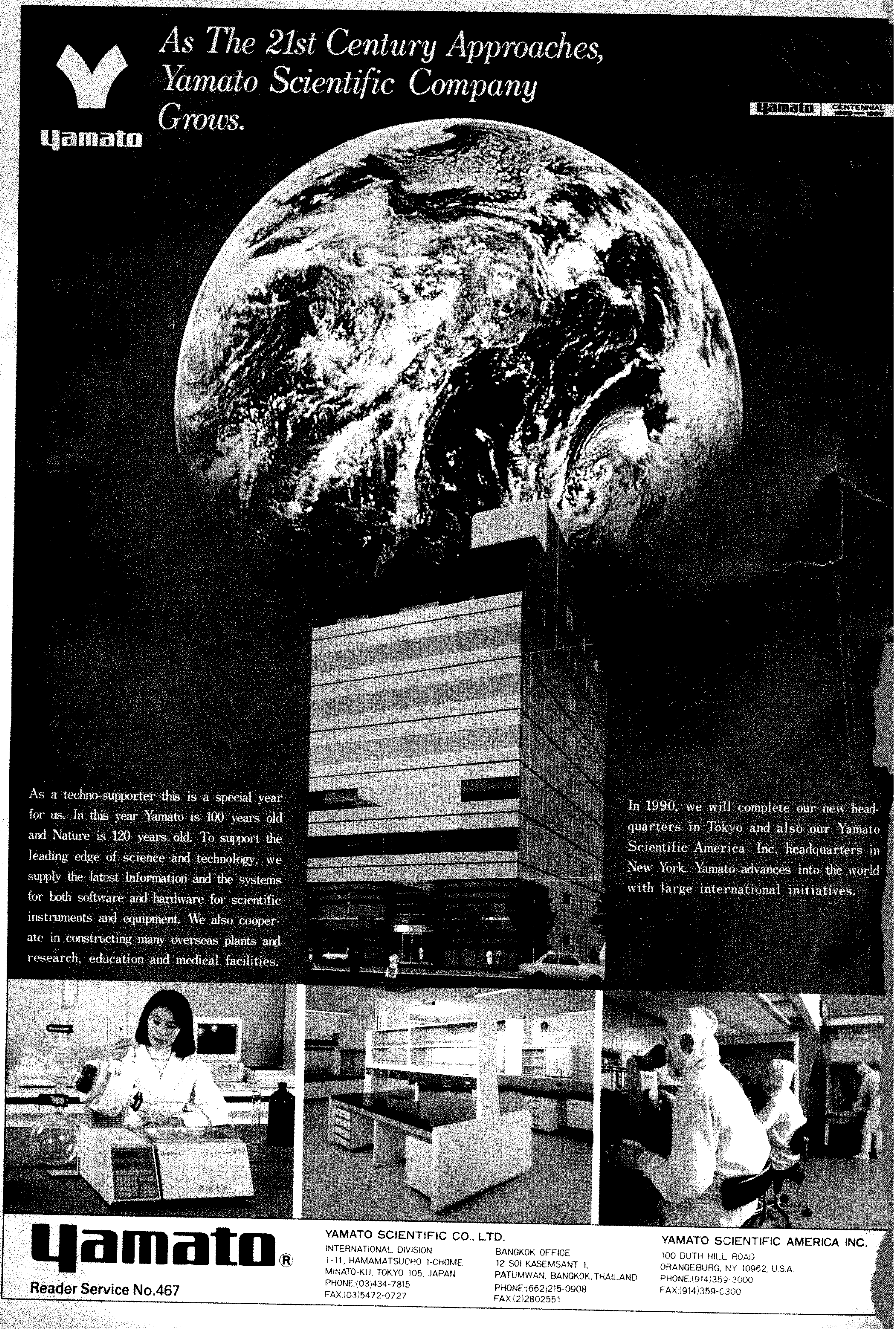

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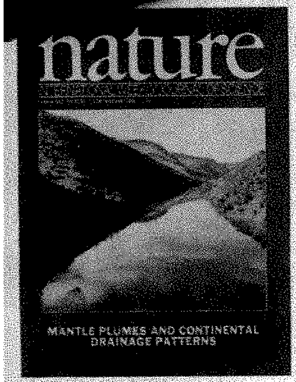

◀ The Rio Pelotas in southern Brazil, along the border between the Santa Catarina and Rio Grande do Sul states. This river flows west, away from the Atlantic, down the flank of a dome of basaltic lavas uplifted by a mantle plume. See page 873.

THIS WEEK

Volcano predicted

A ten-year study of the Poás volcano in Costa Rica provides the first unambiguous evidence that small changes in gravity can be precursors to a volcanic eruption, and may be of predictive value. Pages 902 and 862.

Setback on male gene

Genetic evidence strongly suggests that the development of testes can be determined by a region of the Y chromosome that does not contain *ZFY*, thought to be the testis-determining gene (page 937) but which now seems in fact to determine spermatogenesis (page 940). See also News and Views page 860.

Antibody construction

The hypothesis that a few key conserved residues in the hypervariable regions of antibody genes determine the main chain conformation of these hypervariable loops is supported by structural predictions, see page 877.

Arresting enzyme

Self fertilization in many flowering plants is prevented by self-incompatibility genes with many alleles, but it has not been known how they work. One of these genes was recently isolated from tobacco and is now shown to have strong similarity to ribonuclease. Ribonuclease activity can be demonstrated in proteins extracted from the plant style where they presumably act on the pollen tube cells to arrest growth. Page 955.

Nitrate source

The 'ozone-unfriendly' greenhouse gas N_2O may be produced in the ocean primarily by production of nitrate (denitrification) not, as was thought, by oxidation of ammonium (nitrification). Page 895.

Rules for waves

Experiments performed in a wave tank show that the moderating effect of rain on wave motions is exerted by the suppression of short-wavelength gravity waves, and the accompanying reduction in the breaking of long waves. The finding affects the validity of some remote sensing data, sea surface radar imaging and radar-based wind speed measurements, page 893.

Lunar find

Evidence from a thin section of the Antarctic meteorite Elephant Moraine 87521 shows it to be of lunar origin. But unlike previously discovered lunar meteorites, it is made up of material from a mare region, rather than from the highlands. Page 889.

Signal splicing

Alternative splicing of the mRNA for the D_2 dopamine receptor, the target of antischizophrenia drugs, gives rise to two forms of the receptor, one of which contains an additional 29 amino-acid fragment in a putative signal transduction loop. Pages 923, 926 and 865.

Membrane fusion arrest

When cells enter mitosis, all intracellular membrane traffic is halted. Experiments with endocytic vesicles *in vitro* suggest that the cell-cycle control protein kinase cdc2 is responsible for this inhibition. Page 942.

Enzyme chaperone

Chaperonins, a ubiquitous class of proteins thought to play a part in the post-translational folding and assembly of other proteins, facilitate the reconstitution of the enzyme ribulose bisphosphate carboxylase (Rubisco) in a stepwise fashion from an unfolded state *in vitro*. Page 884.

Guide to Authors

Facing page 958.

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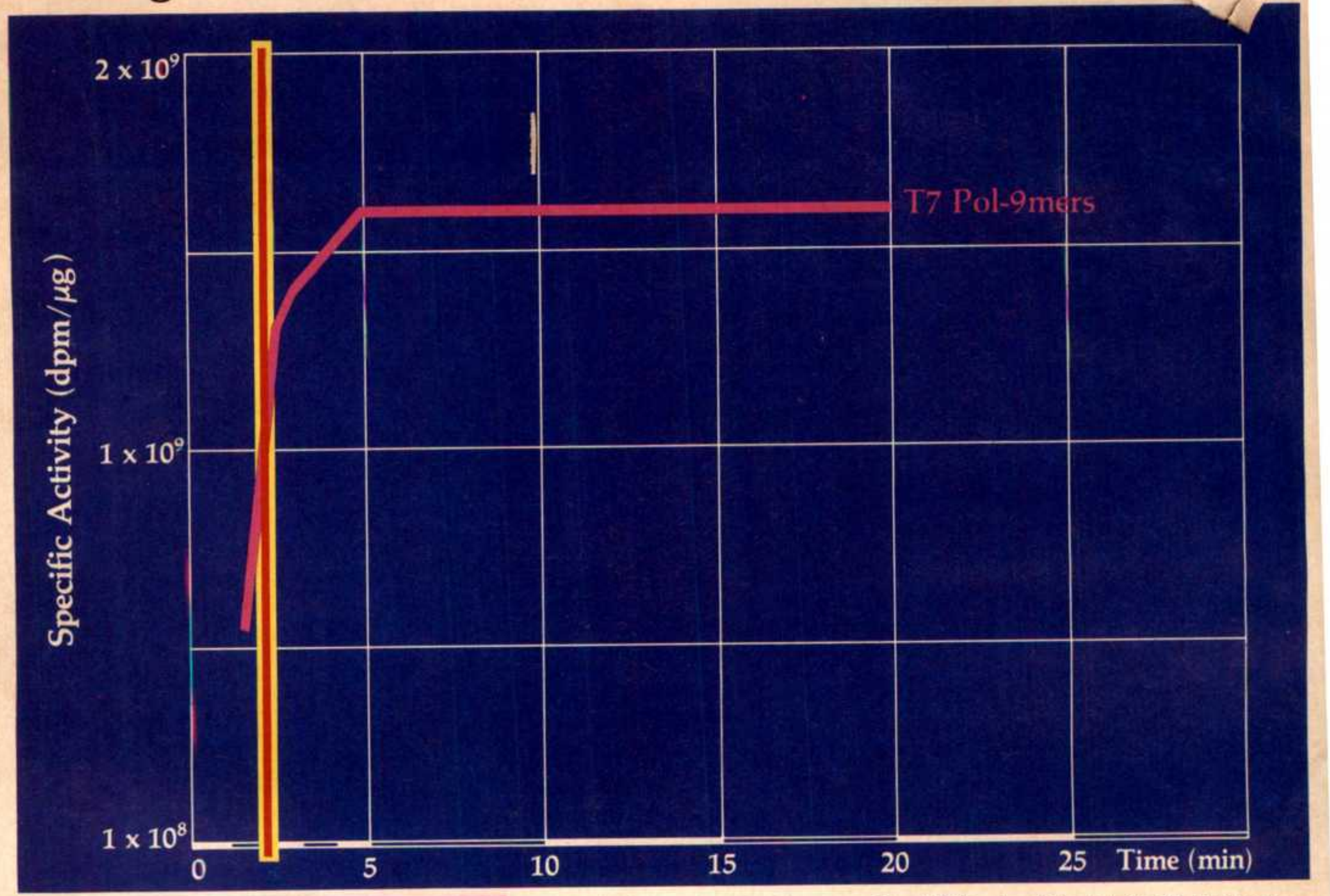
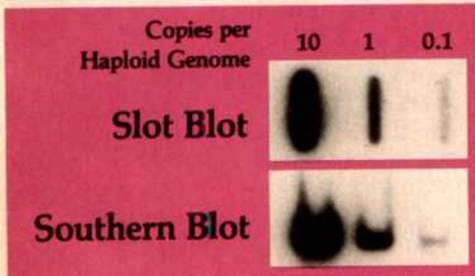
10^9 dpm / μg in 2 M

Using The Prime-It™ Random Primer Labeling Ki





Sensitivity of T7-9-mers DNA probe. The sensitivity of a T7 Pol-9-mers generated probe was performed by diluting the 3 kb template DNA to 3.4, 0.34, and 0.03 pg/μl. Twenty μl of each sample were heated in a boiling water bath for 10 minutes to denature the DNA, then each sample was slot blotted onto a Durlon-UV™ nylon membrane. The DNA was UV crosslinked to the membrane in the Stratalinker™ UV crosslinker. The same quantities of denatured template DNA were loaded onto a 0.8% agarose gel and electrophoresed. The DNA was transferred to Durlon-UV nylon membrane and crosslinked to the membrane as before. Both types of blotted membrane were prehybridized for 30 minutes at 42°C in 5 ml of a solution containing 50% formamide - 1% SDS -1M sodium chloride - 10% dextran sulfate. The T7 Pol-9-mers probe was labeled for two minutes as described above. Following purification of the probe to remove unincorporated nucleotides, 2.5×10^6 cpm of the probe was mixed with 200 μl of 10 mg/ml salmon sperm DNA and then heated in a boiling H_2O bath for 10 minutes. The probe was then added to the nylon membranes in a heat sealable bag and incubated overnight at 42°C. Following hybridization, the membranes were washed for 15 minutes in 2X SSC at room temperature and then for 30 minutes in 2X SSC -1% SDS at 65°C. The membranes were blotted dry and exposed to Kodak XAR film for 48 hours at -80°C with one intensifying screen.

Kinetics of polymerization using Stratagene's Prime-It™ Random Primer Labeling Kit. Incorporation of ^{32}P-dCTP into a 3 kb template was measured by mixing together denatured template, random primers, reaction buffer (containing dATP, dGTP, & dTTP), ^{32}P-dCTP (3000 Ci/mmol) and T7 Pol. Incubation was performed at 37°C for 20 minutes. A one μl aliquot of the mixture was removed at various time points and spotted onto DE 81 filter paper discs. The discs were dried, washed, and the radioactivity counted. Total counts added to the mixture were determined by diluting a 1 μl sample in 99 μl of H_2O and then spotting 1μl of this dilution onto a DE 81 filter disc followed by counting the radioactivity. Percent incorporation was measured and specific activity was calculated.

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Plenary Sessions

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Antigen-Specific Receptors
Fred Alt, Columbia
James P. Allison, UC-Berkeley
Harald von Boehmer, Basel Inst.

MONDAY (evening)
Lynen Lecture*
Philippa Marrack
John W. Kappler
National Jewish Center for Immunology And Respiratory Medicine

TUESDAY (morning)
Distinguished Service Award**
Cesar Milstein

TUESDAY (morning)
Antigen Recognition and Lymphocyte Activation
Jack L. Strominger, Harvard
Richard D. Klausner, NIH
Dennis Y. Loh, Washington University

THURSDAY (morning)
Special Achievement Award***
Wallace Coulter, Coulter Electronics

FRIDAY (morning)
Molecular Mediators of Immune Pathogenesis
Kendall A. Smith, Dartmouth
Thomas Malek, University of Miami
Marian E. Koshland, UC-Berkeley
Eckhard R. Podack, University of Miami
Anthony Cerami; Rockefeller University

Molecular Pathogenic Mechanisms

MONDAY (afternoon)
Diseases of Abnormal Antigen Receptor Formation
Ilan R. Kirsch, NCI-NMOB
Hitoshi Sakano, UC-Berkeley
Terence Rabbitts, MRC

TUESDAY (morning)
Molecular Mechanisms in Autoimmunity
Lawrence Steinman, Stanford
Chella S. David, Mayo Medical School
Bonnie Blomberg, University of Miami

TUESDAY (afternoon)
Disordered Regulation of Lymphocyte Activation
Cox Terhorst, Harvard Medical School
Mark L. Tykocinski, Case Western Reserve
Ira H. Pastan, NIH

THURSDAY (morning)
Genetic Disorders of Surface Molecules
Gerald T. Nepom, Virginia Mason Research Center
Bernard F. Mach, University of Geneva
Yee Hon Chin, University of Miami

THURSDAY (afternoon)
Molecular Mechanisms of Immune-Mediated Tissue Injury
Victor Nussenzweig, New York University
Joan Stein-Streilein, University of Miami
Gilla Kaplan, The Rockefeller University

Biotechnology and the Monoclonal Antibody—The Second 15 Years

MONDAY (afternoon)
Receptors, Immunological Structure, and Pharmaceutical Development
Mark I. Greene, University of Pennsylvania
Donald C. Wiley, Harvard
Jeffrey Greve, Molecular Therapeutics
Eckhard Wimmer, SUNY

TUESDAY (morning)
Manipulating the Monoclonal I
James Larrick, Genelabs
Andreas Pluckthun, Max Planck Institute–Martinsreid
Jorge Gavilondo-Cowley, Center for Genetic Engineering and Biotechnology–Havana

TUESDAY (afternoon)
Manipulating the Monoclonal II
Ellen S. Vitetta, University of Texas
Peter G. Schultz, UC-Berkeley
Christopher L. Reading, M.D. Anderson Cancer Center
Sherie L. Morrison, UCLA

THURSDAY (morning)
The Biotechnology of Allergy
Kimishige Ishizaka, Johns Hopkins
Kevin W. Moore, DNAX Research Institute
Jean-Pierre Kinet, NIH
Tse-Wen Chang, Tanox Biosystems

THURSDAY (afternoon)
New Directions in Immunogenetics
Ursula Storb, University of Chicago
Tak W. Mak, University of Toronto
Charles J. Epstein, UC-San Francisco

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DO, DON'T JANUARY

esponse......... Molecular Pathologies ..

Molecular Pathogenic Mechanisms.
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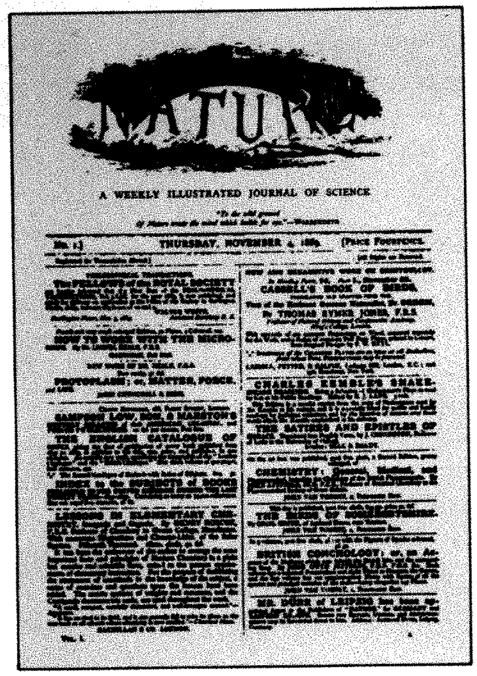

NATURE VOLUME 1 NUMBER 1

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NATURE VOL 342 **nature** 21/28 DECEMBER 1989

Death of a secular saint

Moscow's mourning of the death of Sakharov is understandable, but Sakharov's life has touched all of us in ways that the international research community dares not forget.

IN other times and places, Andrei Dmietrievich Sakharov, who died last week, would have been made a saint. For the past quarter of a century in the Soviet Union, he lived as one. Since his return from exile in Gorky in 1986, the high and mighty have trudged to the tiny kitchen of his Moscow apartment, seeking enlightenment and a little courage. Like the classical saints, Sakharov taught by example, often breathtaking example.

Although all of us everywhere are now in Sakharov's debt, Mr Mikhail Gorbachev is the most conspicuous beneficiary of his turbulent career. The scandalous decision to exile Sakharov was a bankrupt administration's attempt to silence the most distinguished critic of its way of doing business with the courage to speak his mind within the Soviet Union. Exile would have served its purpose if Sakharov had been more easily cowed. In the event, his fortitude during these six years, and his puckish ingenuity in arranging that his opinions continued to circulate, eventually helped to force the election of a reformer as general-secretary of the Soviet Communist Party and then made possible the goal of *perestroika* on which Gorbachev has set his sights. Although Sakharov, pushing for faster change, has latterly been a thorn in his leader's side, Gorbachev did well to acknowledge his debt on Monday morning.

Sakharov's wider contribution to the new temper of Soviet life is the example of his disarming and, often, infuriating directness. His habit was to say what he believed, about personal liberty, nuclear weapons or even the dilemma of Nagorno Karabakh, without calculating in advance what his listeners would think of him for his opinions. Much of the turbulent Congress of People's Deputies now in session has taken its cue from Sakharov. The benefit is that the Soviet people now know how desperate is their plight.

The rest of us have a more subtle debt to pay, arising from Sakharov's sense of professional rectitude. Sakharov was conscripted into military research during the Second World War and, by 1945, had become one of the small group of theoreticians canvassing the feasibility of both the peaceful and the military uses of thermonuclear fusion. As the world knows, the military project was carried through successfully, and Sakharov rewarded with the Order of Lenin (snatched away again when he was exiled). But, from the outset, Sakharov insisted on the dangers inherent in this enterprise. During his own exile to Gorky, he pleaded publicly the cases of others whose plight was even worse, urging that the international community of scientists, "the one real worldwide community that exists today", should shoulder the responsibilities its privileges imply (*Nature* **291**, 184; 1981). How well will the responsibility be discharged now that the prophet is dead? ☐

Page numbers: Vol. 342

LIBRARIANS please note: A production problem caused a discontinuity between the end of the 30 November issue (page 578) and the start of the 7 December issue (page 599). The 'missing' pages, 579–598, have not been allotted.

Leaders lead, OK?

With Sakharov gone, Mr Mikhail Gorbachev will have to change his style.

How should Mr Gorbachev comport himself in the interval between now and the time when there is another gadfly to turn attention from his own subversiveness? He himself must fill the vacuum left by Sakharov. But how?

On one cogent view, *perestroika* has already run into the ground: there is nothing in the shops. At a more abstract level, the rouble is overvalued, meaning that there will have to be a real (not a fake) devaluation in the next few months. People will have to pay more for luxuries, of which they have precious few as things are. Then there may be room for a true price reform, from which even Gorbachev has so far shrunk for fear of the trouble it will cause. Between now and a year from now, Gorbachev has to resolve the economic crisis. So far, luck has been on his side.

The truth, much more encouraging, is that time is also on Gorbachev's side, if only in a narrow sense. So much is apparent from the past few weeks, but here is how that logic goes. Who would have thought, just a few weeks back, that the Soviet enterprise required a new regime in East Germany? Now there is such a one, and also in Czechoslovakia. At this rate, it will be Romania next, or even Albania. For the time being, Gorbachev cannot but be pleased; people, it appears, are on the side of change. But how quickly will change be allowed to happen? Not particularly quickly, it appears. In the short run, Gorbachev will have to face the greater difficulty of saying where he stands. Without Sakharov, that will not be easy. ☐

Moscow bids farewell to the nation's dead hero

■ Academician Andrei Sakharov is buried
■ A city pays its respects

Moscow, Monday

THE nation's grief has never been so all-pervading and sincere as now, when Moscow bids farewell to Academician Andrei Sakharov, the "conscience of the nation". The sense of loss compares in its magnitude only to that at Yuri Gagarin's funeral.

The days that have passed since Sakharov's death on 14 December have shown graphically what profound respect he commanded among the people — this once-defamed man of harsh destiny, the father of the Soviet H-bomb, who spent years in exile under Brezhnev for advocating human rights; the man who bewildered the world with dazzling intellectual revelations and brought it the ideas of new thinking, new relations between different systems; and, finally, the first Soviet holder of the Nobel peace prize.

On 17 December, the temperature in Moscow fell to 20 degrees below zero Centigrade. People fell into long and silent lines along Komsomolsky Avenue where the coffin with Sakharov's body was placed in the Youth Centre. The traditional guard of honour over the body was absent this time: instead, taking shifts every 4 or 5 minutes, were People's Deputies, scholars, Sakharov's colleagues in research and public life, members of the Academy of Sciences of the USSR, poets, writers, representatives of numerous democratic movements and societies.

The ceremony ended at midnight instead of the scheduled 5 p.m. A simple yet moving token of the nation's tribute to the dead academician was a heap of red carnations piled up near the coffin, adorned with funeral wreaths contributed by official and unofficial organizations from all parts of the country.

Today, the ceremony has continued at FIAN, the Lebedev Physical Institute, whence a huge manifestation column set out behind the coffin with Sakharov's body, carried on people's shoulders to the Luzhniki Stadium, where the ceremony became a truly national memorial meeting.

The image of the contentious academician will persist in people's memory. A brilliant physicist–theoretician, he became an academician at 32, the youngest in the Soviet Union. His work on the hydrogen bomb, pioneer ideas on thermo-nuclear synthesis and a number of discoveries in other fields brought him recognition as an outstanding scientist.

He was decorated with the Lenin Order, three Stars of the Hero of Socialist Labour, and given the Stalin Prize — and prohibited from leaving the Soviet Union for reasons of state secrecy.

"Among our scientists, he was, as the saying goes, a transparently-honest man", wrote Nikita Khruschchev in his memoirs. "He hated to think that science might be used for the destruction of life, poisoning the atmosphere and slow killing of the people by nuclear pollution."

Yet it was Khrushchev who failed to heed Sakharov's request not to resume nuclear tests in 1961. This is how Sakharov characterized the ensuing series of H-bomb test explosions: "It was terrible. I became a different man after that." He did not quit science, but concentrated on politics and free thinking, officially called dissent and equated to a crime against the state or a psychiatric disease.

Associated Press

Paying tribute — a long line of Muscovites queue outside the Youth Centre.

It was hard to send Sakharov to a psychiatric hospital and he thus became a symbol of conscience and hope. The desperate letters imploring him for help often bore a simple address: "Academician Sakharov, Moscow". And he undertook to defend the persecuted in the press, in court rooms and in public, even when he realized that his efforts would be in vain.

The Brezhnev system took up the challenge. Without official accusation and trial, Sakharov was sent to the city of Gorky, inaccessible to foreigners for state security reasons. (The exile ended in 1986 with a telephone call from Mikhail Gorbachev.) Academicians denounced him, but he was never expelled from the academy even in the darkest days of witch-hunting, much to the credit of that institution. He stayed having retained his firm perturbed by any persecution

In his book *Reflections on ress, Peaceful Coexistence and Intellectual Freedom*, published in the West in 1968, he called for the elimination of the arms race and the solution of global issues — hunger, racism, disproportionate development of nations and environmental pollution. In 1975 he wrote a book *On Knowledge and the World*. The ideas he developed in that book are obviously parallel to those advanced and discussed in the Soviet Union now.

People's Deputy Sakharov considered *perestroika* to be an event of major magnitude for the Soviet Union and the entire world. He wanted this process to be more speedy and radical in this country. He perceived *perestroika* as a harbinger of the times when the patient Soviet people's long-cherished aspirations and hopes would finally come true.

By his nature, he could not stay aloof from what was going on in his country and abroad, and spoke at the Second Congress of People's Deputies on the day of his death. One of the leaders of the parliamentary inter-regional group, Sakharov was loyal to the end to his absolute and irreconcilable civic stand. The nation will forever remember him as a true patriot, a citizen of his country and the world, a person with an open and generous heart and a fighter for justice and the priority of common human values.

Academician Andrei Sakharov was buried at the Vostryakovo Cemetery in Moscow on 18 December 1989.

Yuri Kanin
Moscow correspondent
Novosti Press Agency

SPACE STATION

A Mir $10 million

Tokyo

THE Soviet Union has some bargains going in slightly used space stations. But do not rush — the Japanese were there first. Last week, in one of the oddest sales of the decade, a tiny Japanese company capitalized at just $7,000 bought the back-up Mir space station for $10 million.

The Horie Group company has only ten employees and usually does nothing more exciting than organize seminars on space. It bought the station while it was on display at World Design Expo 1989, held in Nagoya from 15 July to 26 November. The space station had been on display earlier at exhibitions in France and Canada but there were no takers.

Horie Group says it has no immediate plans to launch the space station but it will allow Japanese companies interested in developing space technology to study it.

David Swinbanks

CANCER DRUGS

Restrictions recommended

Tokyo

An advisory body to Japan's Ministry of Health and Welfare is expected to recommend restrictions this week on the use of the world's two top-selling anti-cancer drugs, Krestin and Picibanil.

The drugs are sold only in Japan and bring in hundreds of millions of dollars each year. But they have become the centre of controversy because of their questionable efficacy.

The Central Pharmaceutical Affairs Council is expected to rule that from now on the two drugs should be prescribed only in combination with other chemotherapeutic drugs and should not be prescribed alone. But a leading critic says the council's decision is unlikely to have much effect on prescription of the drugs and claims that there are serious defects in the process of drug re-evaluation which throw into question the council's impartiality.

Both drugs are so-called biological response modifiers — agents that change the body's response to tumour cells. Krestin is an extract of fungus and Picibanil is a penicillin-treated freeze-dried streptococcus. Both were put on the market in the mid-1970s and their combined sales rose rapidly to about ¥80,000 million ($570 million) a year, which is an order of magnitude larger than sales of anti-cancer drugs in the United States, for example (see Commentary, page 850). Total sales so far amount to more than ¥1 million million ($7,000 million).

Cancer experts have questioned the efficacy of Krestin and Picibanil for years and in 1987 the Ministry of Health and Welfare decided to re-evaluate them. Details of the council's recommendations had not been officially released when *Nature* went to press. But a ministry official confirms that recent reports in the Japanese press of the restrictions to be imposed are "more or less accurate".

Masanori Fukushima of Aichi Cancer Center, who has been campaigning to reform Japan's drug approval system, says the reported decision to allow these drugs to continue to be prescribed in combination with other chemotherapeutic agents represents a "gigantic loophole" . The drugs are already usually prescribed in combination with other anti-cancer agents. And a spokesman for Kureha Industrial Company, which developed Krestin, has confirmed that Krestin is "nearly always" prescribed with other chemotherapeutic drugs.

Fukushima questions the ability of the the council's subcommitee on anti-cancer drugs to re-evaluate Krestin and Picibanil impartially. Fukushima points out that two of the seven subcommittee members carried out some of the clinical trials on Krestin which the subcommittee had to re-assess. And a third member is on the editorial board of the journal *Oncologia*, which published their results. Furthermore, *Oncologia* was established and is financed by Kureha.

A Ministry of Health and Welfare official says that the overlap between members of the subcommittee and those that carried out clinical trials "cannot be helped" because the number of suitable experts in Japan is limited. He says that the root of the problem is the lack of government finance for medical research. He points out that the ministry, which finances the larger part of Japan's medical research, has a budget of only about ¥50,000 million ($350 million) per year, less than one tenth that of the US National Institutes of Health. And so "inevitably" Japanese medical researchers have to seek the financial support of pharmaceutical companies by carrying out clinical trials.

He says that almost all Japanese pharmaceutical companies have their own in-house journals for quick publication of clinical trial results. And while sympathizing with Fukushima's proposal that all clinical trial results used in drug assessment should be published in English in international journals he says this is impractical because of the time it takes.

But Fukushima says that there are plenty of internationally known Japanese researchers capable of carrying out assessment of clinical trials. **David Swinbanks**

ENVIRONMENT

Japan accepts ban on drift-net fishing

Tokyo

Under international pressure, Japan last week agreed to stop using massive fishing nets to catch tuna and squid in the South Pacific Ocean, at least for the time being.

Japan, Taiwan and Korea have been severely criticized by South Pacific island nations, New Zealand, the United States and environmentalists for using drift nets up to 60 km in length in the Pacific Ocean (*Nature* 342, 9; 1989).

Last month, Japan and Taiwan refused to join an international ban on drift-net fishing in the Pacific (see *Nature* 342, 726; 1989). But Japan made a quick about-turn last week to avoid conflict with the United States.

The UN resolution endorsed by Japan last Friday calls for a ban from 1 July 1991 in the South Pacific and worldwide from 30 June 1992. But the ban can be waived if "effective conservation and management measures" are taken. And some environmentalists fear that this may provide Japan with a loophole. David Swinbanks

UK UNIVERSITIES

Selling student places

London

The heads of UK universities last week received details of a new system of competitive bidding for undergraduate places in a letter from the Universities Funding Council (UFC). By June 1990, each university will have to say how many undergraduate places it wishes to make available in each subject area by the 1994–95 academic year, and make an 'offer price' per student for each subject.

Guide prices will be issued within the next few weeks, but the letter notes that "in some (if not all) subjects, universities may wish to . . . improve their chances of having their offers accepted in full by quoting a price below the guide price". Where lower offer prices are made, UFC will leave it to individual universities to show that "adequate arrangements exist for assessing . . . academic standards".

After assessing the bids, UFC will inform each university by early 1991 of the number of student places to be supported and the prices for teaching that have been accepted for the 1991–92 academic year, together with provisional figures for the three following years. As well as cost and teaching quality, the UFC says the range of subject provision and the balance between different subjects will be taken into account in the allocation of student places and teaching grants to the universities.

Some subject areas are excluded from the competitive bidding system. Student numbers for medicine, dentistry and veterinary science will be allocated to universities by the UFC and supported at the relevant guide price.

The new system reflects the government's desire for a more market-orientated university system. Sir Peter Swinnerton-Dyer, chief executive of UFC, says that the new arrangements will "increase institutional autonomy and widen student choice as the balance of public funding for higher education moves from centrally provided grants to fees".

The government expects student numbers to increase by just under 10 per cent between 1988–89 and 1992–93, and for there to be a shift towards science and technology. UFC expects an increase of at least 10 per cent by 1994 – 95 but the exact figure will depend on the universities' bids.

The Committee of Vice-Chancellors and Principals (CVCP) was concerned at its meeting last Friday that funding for the universities for 1990–91 may not be sufficient to provide "satisfactory pay settlements". It has also announced the formation of an 'Academic Audit Unit' to review each university's mechanisms for monitoring academic standards. A full statement on the competitive bidding system is now epected in mid-January, after the next CVCP meeting.

Peter Aldhous

ANIMAL RIGHTS

German research fights back

Munich

THE naming of the first "animal protection official" in West Germany has mobilized some of the country's top researchers in defence of the use of animals in research. Although protests from researchers have already led to limits on the power of the new official, the creation of the post reflects the growing political power of animal rights activists. Important changes in the way research with animals is regulated and monitored by the state may follow.

Researchers were so confident of their postion that they supported Minister-President Walter Wallmann (Christian Democrat) of the *Land* of Hesse when he created the new post. Researchers felt that their right to do experiments using animals was effectively protected by West German law.

Every experiment must be approved by a committee consisting of a two-thirds majority of 'experts' from the fields of medicine, biology and veterinary science.

But the man Wallmann nominated, the journalist and animal rights activist Ilya Weiss, was seen as the last person researchers wanted in the job. Researchers are now worried that the fashion of having an 'animal ombudsman' may spread to other *Länder* or to the federal government.

Weiss, who is 36 years old, is due to begin his duties on 1 January. He has been one of the most ardent advocates of the animal liberation movement in West Germany and publicly supported such actions as the 'freeing' of macaques from the research unit of INSERM in Lyons (see *Nature* **339**, 407; 1989). Weiss was the head of a West German animal rights organization, and has conducted a 14-year crusade against the abuse of animals. He once locked himself in a huge cage in a central Frankfurt square for several days in order to dramatize the plight of animals used in research.

Weiss will have two full-time employees and an estimated budget of DM500,000 ($290,000). His official task will be to observe the application of the West German Animal Protection Law in Hesse and to suggest improvements in the interest of animals in both agriculture and research. He will act as an agent for Hesse in animal protection matters on both a national and a European level.

On 30 November, Wallmann met with representatives of the employees' committee at Hoechst AG, the pharmaceutical manufacturer, which is based in Hesse. On 7 December, he met more than a dozen senior researchers and doctors, some of whom came from other *Länder* to warn him of the possible consequences of Weiss's nomination.

Wallmann assured both groups that Hesse would remain an attractive location for research and industry and that he did not intend to limit animal experimentation "as long as it is necessary". Furthermore, he assured researchers that Weiss would have to clear all of his public statements with the government.

Researchers were disappointed, however, that Wallmann did not forbid Weiss access to the documents that researchers must file in order to gain permission to carry out experiments with animals. They fear that Weiss will pass on the contents of the files to animal-rights activists who might attack individual researchers.

Although animal-rights activity receded after the Animal Protection Act was redrafted by parliament in 1985, the potential for activity is still present — the national animal rights organization 'Deutscher Tierschutzbund' has 600,000 members, about one per cent of the population of West Germany. Peter Gutjahr-Löser, who follows the issue for the Max Planck Society, foresees an upsurge of activity.

A spokeswoman for the granting agency Deutsche Forschungsgemeinschaft (DFG), said that DFG preferred to wait and see what else happens in Hesse before taking any public action. It is important, she said, to see if Weiss does his job properly before responding.

Researchers are especially worried that they would lose support if they are forced to fight in public for the cause of animals experimentation instead of just in private meetings with politicians. "If we had to go up against Weiss in a television debate, we would lose", said Wässle, because "we are amateurs and he is a trained professional." Weiss makes blanket statements condemning the use of animals, whereas researchers are trained to explain complicated issues in a qualified and cautious way, said Wässle. There is no doubt about which method comes across better.

Wässle rejected tactics such as sending leaflets to all the physicians in the country asking them to make their patients aware of the medical need for animal experiments. Although such tactics have been used by researchers in the United States, they would be seen as exploitation of the patients in West Germany, he said.

But not all the news is bad for researchers. The history of disagreements within the animal rights movement may reduce the threat to research. Some activists opposed the nomination of Weiss to the post in Hesse because they felt he was too radical.

Gutjahr-Löser admitted that Weiss's nominations "could have some positive effects" by raising the consciousness of researchers about animal rights. But researchers "do not need Weiss to make them think".

It would make more sense, he said, to hire more experts and veterinarians for the animal protection bureaucracy in order to speed up the application process, which has begun to fall far behind the timetable guaranteed by the law, and to increase vigilance against possible abuse of the animals.

Steven Dickman

ANTARCTIC RESEARCH

New Indian base planned on Weddell Sea

New Delhi

INDIA has despatched an expedition team on a chartered ship to the coast of the Weddell Sea in Antarctica with the eventual aim of establishing a new permanent base in that region. Known to be rich in mineral resources, it lies in an area where Britain, Chile and Argentina have overlapping territorial claims.

The 21-member expeditionary team, which left on 4 December, is led by Dr B.K. Raina of the Geological Survey of India. The team includes five scientists and 16 members of the Army, Navy, and Air Force. This is the ninth expedition to Antarctica since 1982, when the Department of Ocean Development (DOD) set up a station named Dakshin Gangotri at 70 degrees south and 12 degrees east. The station, located on shelf ice, began to drift and in 1986 the Indian team set up a second camp about 80 km inland. A permanent base has now been established there.

The expedition to the Weddell Sea area is a sign that India's Antarctic activities will diversify. Vinod K. Gaur, the new Secretary of the Department of Ocean Development says the team will conduct geological studies, and that the department will eventually establish a permanent station there.

India is a consultative member of the Antarctic Treaty and has been a member of the Scientific Committee on Antarctic Research since 1984. So far, the Department of Ocean Development has spent about $30 million on its eight expeditions.

The department intends to purchase a ship for future expeditions and has asked the government for about $60 million for Antarctic research between now and 1995.

India does not recognize the territorial claims in Antarctica of any countries and says its present goals in Antarctica are purely scientific. But it has been stated officially that economic benefits for India can come from knowledge of the krill stock, and a fisheries vessel capable of operating up to 60 degrees latitude is being acquired.

K.S. Jayaraman

PATENT LAW

Talking of harmony

Washington

1989 ends with patent lawyers hopeful that at long last the holy grail of patenting — the international harmonization of patent laws — lies within their sight, if not yet within their grasp. The reason for this surge of optimism is the unexpected success of the World Intellectual Property Organization (WIPO) set up last year in Geneva under UN sponsorship.

Unlike two earlier organizations that attempted harmonization of patent law, WIPO involves 121 countries, not just the patent offices of Europe, the United States and Japan. WIPO sponsored a successful meeting in Geneva in November at which patent experts examined the details of a draft harmonization treaty. It will sponsor a second meeting next June, with the aim of preparing a treaty for a diplomatic conference in June 1991.

The draft treaty that WIPO discussed in November would bring in some striking changes. The most contentious for the United States is a change from a 'first-to-invent' system to the 'first-to-file' system practised in Japan and Europe in which the patent is granted to whoever wins the race to the patent office. Although proponents of the US system contend that it is fairer, the procedures to determine who was first to invent are time-consuming and expensive.

Many of the clauses in the treaty are hotly disputed by all WIPO members, but one that meets with few obstacles is the plan for a uniform 'grace period' — the time before a patent application is filed during which the disclosure of a competing invention cannot influence the granting of a patent. The United States allows a grace period of one year and Japan six months but Europe has no grace period at all. This difference is one of the most troublesome for those filing for patents in several different countries. Most members agree on a 12-month grace period.

A rule that patent applications must be published no later than 18 months after they are filed is strongly supported, with objections only from the United States and New Zealand. The United States complains that it is unfair to publish a patent application because if the patent is not subsequently granted, valuable secrets may be given away. As the US patent office grants patents, on average, about 18 months after the date of application, the United States argues that there is no need for compulsory early publication, especially as much of the information is disseminated in scientific jurnals. But Japan argues that early publication benefits both everybody because it avoids a long period of insecurity during which the contents of patent applications remain unknown.

Japan's preference for early publication is in part explained by the remarkable length of time it takes to examine patents. Not surprisingly, Japan objects to attempts to put a time limit of 18 months on examination of patents. Opposing the United States, United Kingdom, the Soviet Union and others supporting the limit, Japan argues that strict time limits have no place in an international treaty because many unpredictable factors, such as surges in the number of patent applications or a shortage of patent officers, could block even efforts made in good faith to meet them.

One article in the draft treaty which could reduce the enormous number of patents currently clogging up the Japanese system gains universal support. The article broadens the meaning of 'unity of invention', so that technically related features of an invention are all protected under one patent, rather than under several different ones as is now common.

The question of patent lifetime provokes much argument. The draft WIPO treaty would provide 20 years protection. Uruguay is among those that argue that 20 years is too long, and India argues that the term should vary for different fields of

EPO PATENT DISPUTE

Court battle ends at the start

Washington

One of biotechnology's longest-running battles was expected to end last week but now looks set to go into a new round of bargaining and bickering. On Monday 11 December a Boston court ruled that neither of the two protagonists — Genetics Institute of Boston and Amgen Corporation of Thousand Oaks, California — has a clear overall right to produce recombinant erythropoietin (EPO) by genetic engineering. In the short term, the ruling seems to favour Genetics Institute. EPO is a kidney cell protein that stimulates bone marrow red-blood-cell production, and annual worldwide sales are expected to reach between $250 million and $1,000 million.

The ruling, which is complex and runs to 184 pages, validates the central claims of the patents held by the two companies but at the same time doubles the confusion by considering both patents partially invalid and mutally infringing.

The Genetics Institute patent, issued in June 1987 after the company purified EPO from human urine, refers to "homogeneous human EPO", a term that the company claims to include recombinant EPO. The Amgen patent, issued in October 1987, covers the gene, vectors and cells required for production of recombinant EPO, although the patent did not specify the product itself.

Genetics Institute's version of recombinant EPO is called Marogen and is currently only in experimental use in the United States as it has not gained Food and Drug Administration (FDA) approval.

Amgen's recombinant EPO, called Epogen, gained FDA approval in June, 1989 and already has sales of more than $50 million.

The most immediate effect of the court decison may be to make it easier for Genetics Institute to produce Marogen abroad. The Amgen patent has so far prevented Genetics Institute from manufacturing recombinant EPO in the United States. But it has been getting around the restriction by licensing Marogen to Chugai Pharmaceuticals of Japan and importing the product into the United States.

The new ruling found that offshore production of recombinant EPO by Chugai Pharmaceuticals does not infringe the Amgen patent, effectively putting an end to legal action by Amgen designed to prevent the import of Marogen. Even worse for Amgen, the ruling says that Amgen cannot produce Epogen in the United States without infringing the Genetics Institute patent. That puts Amgen in the same boat as Genetics Institute, which cannot produce recombinant EPO without infringing Amgen's patent. Amgen says that the ruling is unlikely to affect the availability of Epogen to patients. The stage looks set for a tussle over some form of cross-licensing agreement.

But Amgen may try to gain an advantage by waiting for the outcome of actions associated with a wider EPO dispute. Amgen has submitted two EPO process patents. If they are granted, Chugai Pharmaceuticals would be prevented from importing recombinant EPO into the United States.

Amgen is also benefiting from 'orphan drug' status for Epogen, granted by FDA for the treatment of anaemia in patients with chronic renal failure. This provides Amgen with a seven-year monopoly in the United States (see *Nature* **339**, 493; 1989). Marogen, which is awaiting FDA approval for sale in the United States, could receive equivalent status if the FDA declares it to be a product distinct from Epogen. If not, an agreement may be negotiated whereby the orphan-drug status of Epogen is shared with Genetics Institute in return for Amgen not paying royalties in a cross-licensing agreement.

Both parties may, however, wish to see a speedy resolution to the dispute as the market for EPO is huge. **Diane Gershon** technology. The decision on patent lifetime will not rest with WIPO, however, but with GATT (General Agreement on Tariffs and Trade), which will be considering harmonizing the term of patent protection in discussions of intellectual property rights next year.

Developing nations will be anxious for terms that recognize their special circumstances — the need to have access to sophisticated drugs at reasonable prices, for example. They intend to meet in Geneva before the June WIPO meeting in order to thrash out a common position on the treaty.

Christine McGourty

AIDS

East Berlin risks grows

Munich

TANGERINES and tape recorders are not all that East Germans are bringing back from their trips to West Berlin. Unless East German citizens take precautions, AIDS may be the next big import from the capitalist West. That is the view that emerged when East German public health official Niels Sönnichsen visited an AIDS self-help group in West Berlin.

Sönnichsen, who is at the East German Ministry of Health, and his counterparts in West Berlin have begun an information and condom distribution campaign. The campaign is unprecedented in East Germany, where AIDS was a taboo subject until just a few weeks ago. It is aimed at homosexual men, the only group currently at high risk for AIDS in East Germany. The main message: practise safe sex while in West Berlin.

With 16 confirmed cases of AIDS and seven deaths, East Germany has always been one of the most AIDS-free countries in Europe. By contrast, West Berlin, a city of about 2 million, has had 882 AIDS cases and 349 deaths, according to the West German Federal Health Office. The number of AIDS virus carriers in West Berlin is estimated to be between 5,000 and 10,000. East Germany, with a population of more than 16 million, is thought to have 200 to 400 carriers.

But many of the West Berliners infected have been intravenous drug users, as many as 40 per cent of whom are thought to carry the virus. As there is virtually no drug trade in East Germany, only homosexuals are thought to be at risk there.

A self-help group, Berliner AIDS-Hilfe, has distributed condoms at homosexual meeting places in West Berlin since the wall was opened. The next step, said spokeswoman Birgit Thomas-Schön, is to give doctors in East Berlin training in diagnosis and counselling. But AIDS cannot be stopped at the border, says Meinrad Koch, head of the AIDS centre of the Federal Health Office in West Berlin.

Steven Dickman

SPACE SHUTTLE

Rescue mission prepared

Washington

THE mission of space shuttle Columbia, scheduled tentatively for launch on 8 January 1990, will be the first occasion on which the shuttle performs a task that was originally intended to have been one of its routine activities — the return to Earth of a satellite put into orbit on a previous shuttle flight. If all goes well, the crew of Columbia will manoeuvre the shuttle next to a cylindrical canister known as the Long Duration Exposure Facility (LDEF), pull it into the cargo bay, and bring it back down to Earth, where scientists will see the outcome of 66 experiments that have been sitting in the cold and the dark for five and a half years.

LDEF, put into orbit in April 1984, is a 12-sided cylinder, 30 feet long and 14 feet in diameter, holding 86 trays that contain a variety of simple experiments in physics, astronomy and biology. It was supposed to stay in low orbit for about a year, but before it could be retrieved, the Challenger explosion of January 1986 grounded the shuttle fleet for three years. This week's [planned] flight is an eleventh-hour mission: Columbia is the only shuttle with a cargo bay big enough to hold LDEF, and if the retrieval attempt fails, LDEF, now in a rapidly decaying orbit, will burn up in the Earth's atmosphere, probably by February next year.

The unexpected length of LDEF's flight, and the consequently aggravated effects of the upper atmosphere on its contents, could turn out to be the biggest problem for some of the experiments on board, according to Ernst Zinner, of Washington University in St Louis, Missouri, who is principal investigator of an experiment to capture interplanetary dust. Zinner's payload on LDEF consists of a number of 'sandwiches' of germanium plates in metallicized plastic foil coatings. Dust particles, travelling at speeds of 10 km per second, penetrate the foil but crash into the germanium and vaporize; the lower-speed fragments should then be retained by the foil coatings, so that the sandwiches retrieved from LDEF should harbour a five-year accumulation of deposited atoms whose isotopic composition will offer clues as to the origin of the dust. But Zinner is worried that some of his germanium sandwiches, placed in trays at the leading edge of the LDEF, will have been poisoned or damaged by the long exposure to atmospheric oxygen, in which case the germanium will contain an inseparable mix of terrestrial and extraterrestrial atoms.

But the unexpectedly long exposure of LDEF to the elements of space should also bring some rewards. Many of the experiments on board were designed to assess the damage suffered by a variety of materials on long exposure to dust, cosmic rays and meteorites, and the body of the LDEF canister itself will be examined closely for pitting and other ill-effects. This whole investigation will add greatly to the sparse body of knowledge on the durability of man-made structures in space, and should provide food for thought for the engineers now designing the space station.

One final LDEF experiment will give schoolchildren across the United States the chance to participate in a little space research. The George W. Park Seed Company of Greenwood, South Carolina, provided 12.5 million tomato seeds in packages loaded on to the LDEF for the Space Exposed Experiments Developed for Students (SEEDS), and set aside another 12.5 million which have been maintained in a sealed environment on the ground since April 1984. The tomato seeds will be divided up into kits of 50 exposed and 50 control seeds, and will be distributed to 250,000 schools as well as to some universities. Students are then free to investigate the effects of space exposure how they choose; young schoolchildren will be able to compare germination rates and times, while university students can look for genetic changes. **David Lindley**

RESEARCH COUNCILS

NERC on the up

London

A "RESURGENCE" in the fortunes of the UK Natural Environment Research Council (NERC) after years of financial attrition, is the optimistic message of the council's report for the year ending 31 March 1989. At a press conference to publicize the report, NERC chairman Professor John Knill described how a 20 per cent rise in government funding over that for 1987–88 reflected "a dramatic change in public and political attitudes to environmental issues".

But the increase in resources available to NERC since the science budget announced in November 1988 did not prevent a 7 per cent cut in staff numbers over the year, a hangover from earlier financial problems. This year's science budget has yet to be allocated among the research councils.

The report also comments on proposals to reorganize the administration and funding of UK environmental research. NERC is opposed to a full merger with the Agriculture and Food Research Council (AFRC), first suggested by the House of Lords select committee on science and technology in October 1988. Nevertheless, there is "considerable logic in a partial merger between AFRC and NERC", perhaps within a division of a single National Research Council. Peter Aldhous

Journals and databanks

SIR—Recent discussions in these pages ("Who's hiding the primary data?" *Nature* **341**, 94; 1989 and "Making good databanks better" **341**, 277; 1989) have praised the goals of the biological databases, pointed out some problems with them, but rejected a role for journals in ensuring their completeness. While we welcome your enthusiasm for our goals, the arguments against the role for journals fail to take account of the facts.

Insistence on deposition of data is seen by you as an unreasonable burden on authors: journals should not be watchdogs; there should be as few obstacles between the author and publication as possible; political, economic and technical restrictions render access to the databases patchy, and it is unreasonable to insist that scientists contribute to a resource that serves them only poorly. Arguments about the workability (there are so many databases nowadays) and enforceability of deposition schemes are also raised.

That journals are watchdogs is already a fact — non-trivial publication standards and review procedures are already in place to ensure the scientific integrity of what they publish. That these procedures are an obstacle between the author and publication does not, of course, justify the creation of others, but the deposition of sequence data is a necessary part of ensuring integrity. It is now common for articles to discuss sequences or structures not actually presented, and even presented sequences would be more accessible in a computer database. Readers cannot assess conclusions based on data they cannot see. Your leading article "Making good databanks better" even stresses that journals should ask for and make available such supporting data as are appropriate. What better way is there to do that than through the centralized databases?

That access to the databases is uneven is true. Any implication that we complacently direct our efforts to the service of a high-tech subcommunity is not. For some less well equipped users, we produce tape formats long-since obsolete. Our CD-ROM is targeted for users in low-investment computer environments. It also contains search software that may help to obviate the need to purchase expensive third-party products.

Nonetheless, inequalities exist: but nor are journals, libraries and scientific equipment equally accessible to all. The decision not to include the Soviet Union in the present tripartite nucleotide sequence database collaboration was based purely on a notion of diminishing returns with increasing numbers of collaborators. We routinely exchange data with them.

The databases, again like journals, libraries and scientific equipment, are as accessible to commercial organizations as to academic researchers. Publication of scientific findings (in databases or journals) renders them available for exploration. For the sequence database, this is no bad thing. The development of software tools for exploring genome data is in its infancy. Maintaining the complete data collections in the public domain allows any group — commercial or academic — to carry out research in this area and promotes a diversity of approaches appropriate at this stage.

The workability and enforceability of our data deposition systems is no longer a matter of debate, a number of journals already operate such systems with total compliance, and our feedback from editors and authors is positive. Also, some attempt to minimize the problems of dealing with multiple databanks is being made. The United States, Japan and Europe all have nucleotide and protein sequence databases (incidentally the DNA Database of Japan is at the National Institute of Genetics in Mishima, not the Riken Laboratory as you suggest). These six sequence databases have agreed to deal with journals in a coordinated manner.

Insofar as the databases are pleading for data, they are reflecting the demands of the scientific community. It is the users — publishing scientists themselves — who need the data. We do all in our power to ensure the completeness of our collections, but the task is enormous. Without the support of the researchers at large, we will serve them poorly. For unpublished, possibly confidential data, deposition can be urged. For published data, our goals are compatible with those of the journals. Network access to our databases has been possible since June 1988; it is not, as you suggest, a thing of the future. Readers of journals enforcing deposition can typically access the data on the day they receive the journal. The two information resources work well together.

On receipt of reasonably documented nucleotide sequence submissions, we issue accession numbers within one week. They are proof of deposition and an unchanging pointer to the data. We feel it would be appropriate for *Nature* to insist that such numbers be presented at some time in the publication process.

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MS processing

SIR—*Nature* has done a service to authors in an earlier issue this year by publishing the median times in which it accomplishes each stage of manuscript processing. I hope that other journals will take up the same practice. What would be desirable is that all journals not only report on this matter periodically as *Nature* proposes to do, but also reprint the essential statistics at last compilation on the same page or pages where they give their instructions to authors, page charges, reprint prices and so on. A would-be author could then easily find the information when he especially needs it: that is, when considering the advantages and disadvantages of submitting to a particular journal.

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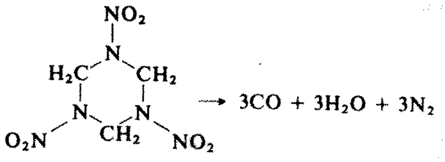
How not to defeat terrorism

SIR—Robert Michaels' suggestion (*Nature* **342**, 336; 1989) that the problem of terrorist bombings of airplanes might be solved by the exclusion of oxygen from the cargo holds of aircraft is ingenious but I am afraid it will not work. Modern molecular explosives, such as TNT, PETN and RDX, do not require oxygen for their detonation but instead contain both oxidant and fuel within the same molecule. For example, the military explosive RDX decomposes on detonation according to the following scheme:



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SIR—Robert A. Michaels (*Nature* **342**, 336; 1989) proposes to suffocate terrorist bombs by flushing aircraft luggage bins with nitrogen. DREADCO's torpedo experts assure me that explosives are immune from suffocation. They can explode quite happily in nitrogen, or indeed under water. Human beings, however, are not so self-contained. Michaels' system would merely expose the hapless passengers to a new risk — that of suffocation from nitrogen-leaks. On the bright side, however, it would certainly discourage smokers.

DAEDALUS
University of Newcastle upon Tyne, UK

Letters submitted for Correspondence should be typed, double-spaced, on one side of the paper only.

Dangerous chemicals

SIR—Wahlström[1] recognizes the ecological and health problems caused by a great number of existing chemicals and suggests a phasing-out procedure for a 'sunset' list. West German activities in this area may be relevant.

In 1982, an Advisory Committee on Existing Chemicals of Environmental Relevance (BUA), consisting of representatives from science, industry and government, was established at the Society of German Chemists (GDCh). Under the German Chemicals Act, existing chemicals must be reported to the competent authority if they exhibit properties indicating they may be hazardous, either alone or in combination with other chemicals (§ 4(6) ChemG). The goal of that committee is to seek appropriate solutions for dealing with chemicals relevant to health and to the environment by the adoption of voluntary measures. The committee selects and examines existing chemicals for environmental or health purposes as authorized by the German Chemicals Act, using exclusively scientific criteria.

The estimated number of about 100,000 chemicals in the European Inventory of Existing Commercial Substances (EINECS) prevents selection on the basis of strictly quantitative criteria. Even if the necessary resources were available, reliable data for a quantitative evaluation are lacking in most cases.

On the basis of the limited data available, a pragmatic approach has been developed for selection. To this end, a list of chemicals was compiled from various priority lists: the presence of these chemicals in the environment has been proved, or is highly probable; they are industrially important, or are manufactured in large quantities. Approximately 4,500 chemicals were selected in this way, of which about 1,000 were selected because of their occurrence in the environment and their industrial relevance.

After elimination of chemicals subject to special legal regulations, materials of natural origin, inorganic chemicals and chemicals that are unstable in the environment, 512 substances remained. For these substances, data were collected according to eight selection criteria covering environmental exposure and effects. These data were scored in a scoring procedure for selection purposes. The result was a list of 60 chemicals that may be of environmental relevance and which consequently should be treated with priority[2].

In continuation of this work, a second list of 75 environmentally relevant chemicals was published[3]. There, the production volume of these chemicals served as an additional criterion. The second list is associated with considerable uncertainty because of the partial incompleteness of the data and because their scientific quality could not be checked individually in all cases. This means that, as with the chemicals on the first list, the second list may also include substances that are not hazardous to the environment, and also that some environmentally hazardous chemicals have been omitted. Consequently, a conclusive evaluation is feasible only if all available data have been collected in separate substance reports and if data that may be lacking have been obtained by appropriate testing.

Meanwhile, about 40 such individual substance reports have been published and about 80 reports are being discussed or about to be published. The importance of these substance reports can be illustrated by an example. In the case of pentachlorophenol (PCP), production in West Germany has been shut down voluntarily as a consequence of the report because PCP is considered as a major source of dioxins in the environment[4]. It is disappointing that companies in other countries have not followed these recommendations, but have even increased their production. As long as non-ecological behaviour leading to more profit governs our decisions, no solution to our environmental problems can be expected.

A third priority list is in the process of being evaluated by an *ad hoc* working group of the advisory committee. In addition, regular scientific symposia provide a platform for the presentation and discussion of the results to the scientific community and the public. Two such symposia have so far been organized under the auspices of the Society of German Chemists.

In conclusion, the commendable five-step phasing-out procedure suggested by Wahlström has already been realized on a national basis in the West Germany. The selection criteria and priority lists developed by the Advisory Committee on Existing Chemicals of Environmental Relevance could also serve as an international model.

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1. Wahlström, B. *Nature* **341**, 276 (1989).
2. GDCh-Advisory Committee on Existing Chemicals of Environmental Relevance (BUA) *Existing Chemicals of Environmental Relevance. Criteria and List of Chemicals* (VCH, Weinheim and New York, 1989).
3. GDCh-Advisory Committee on Existing Chemicals of Environmental Relevance (BUA) *Existing Chemicals of Environmental Relevance II, Selection Criteria and Second Priority List* (VCH, Weinheim and New York, 1989).
4. Beratergremium für umweltrelevante Altstoffe (BUA), *Pentachlorphenol. BUA-Stoffbericht 3* (VCH, Weinheim, 1986).

Counting partners

SIR—It is true that the average (lifetime) number of heterosexual partners per person should be the same for men and women (*Nature* **342**, 12; 1989), but this will be apparent only if the total population is known. Concentration of promiscuity in a small minority of women will cause most samples to show a wide difference between the sexes.

To take a hypothetical example: suppose a population of 10,000 men and 10,000 women in which 9,990 of the women and 1,000 of the men have been faithful to their partners, and in which the remaining 10 women have earned their living by making themselves available to each and every one of the remaining 9,000 men; almost all samples will show the average number of partners as one for the women and ten for the men.

It should not be supposed that most informants have lied but that researchers have had difficulty in finding sufficient numbers of highly promiscuous women. All the more reason for expanding the research!

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Embryo research

SIR—The dilemma facing the British government in implementing the Warnock recommendations[1] is that, if experimentation with or destruction of artificially fertilized embryos is prohibited after 14 days, anti-abortionists could demand the same protection for naturally fertilized embryos. One solution is to scrap the arbitrary 14-day limit.

The German government has chosen a second solution. It is about to legislate against any embryo research which is not for that embryo's own benefit. In so doing, the German government is acting on the Nuremberg Codex developed after the Nazi war-crimes trials[2]. Should this not give us pause?

Certain German scientists and doctors rationalized, supported and even participated in the Holocaust[3,4]. This fact is now universally abhorred. But how many of us would not have been among them, given normal concerns for personal and family security and also career pressures? If this is so, we must now ask how many of today's scientific lobby for embryo research are free of such concerns and pressures. If not thus free, may not today's lobby be equally mistaken?

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1. *Nature* **341**, 673 (1989).
2. Dickman, S. *Nature* **333**, 791 (1988).
3. Müller-Hill, B. *Murderous Science* (Oxford University Press, 1988).
4. Seidelman, W.E. *Nature* **337**, 300; **341**, 180 (1989).

The overdose of drugs in Japan

Masanori Fukushima

The Japanese consume enormous amounts of drugs on prescription, some of which are not legally available elsewhere in the world. The reason is a defective national system for drug approval and dispensing.

THE Japanese are the world's highest spenders on prescription drugs. Among the top-selling drugs are anti-cancer agents peculiar to Japan that are of questionable efficacy and yet have sales of hundreds of millions of dollars each year, far more than the sales of anti-cancer drugs in other developed nations. Moreover, several of the drugs sold only in Japan have serious side effects. Justification of these contentions is to be found in Tables 1–3.

What has caused this worrying situation? And how can drugs be million-dollar sellers in Japan and yet not sell elsewhere in the world? The answer is that there is a whole series of defects in Japan's drug-approval process and in the system for dispensing approved drugs.

At first sight, Japan's system of clinical trials resembles that of some other countries. In principle, drugs must pass through three phases of clinical trials as, for example, in the United States. Phase 1 is to check a drug's safety in humans; phase 2 is to assess its therapeutic index (the response rate and the severity of side effects) in selected populations of patients for whom the drug is intended; and phase 3 is to determine whether the new treatment is better than existing ones. In the case of anti-cancer drugs, however, once a drug has passed phase 2 it can be widely prescribed throughout Japan. The phase 3 clinical trials in effect become part of the pharmaceutical company's marketing strategy. Further, they do not meet the rigorous requirements of such trials elsewhere in the world — they tend to assess effect only (reduction in tumour size, for example), which is measured during phase 2 trials, rather than benefit (risk-to-benefit, and cost-to-benefit), which is measured during phase 3. So drugs which have some effect but are of little proven benefit can reach the market.

A further disturbing feature is that patients are often given drugs under trial without being asked for their informed consent — that is, without it being explained to them that the drug is experimental, or obtaining their signature on a specific document outlining certain details about the particular study and acknowledging the patient's willingness to participate in it. In the United States and other developed countries, patients involved in clinical trials must fill out and sign detailed consent forms. Thus many drugs that sell well in Japan cannot be sold elsewhere because satisfactory phase 3 trials have not been carried out.

In contrast with conditions in countries such as Britain, where doctors are not allowed to sell drugs, nearly all Japanese general practitioners and hospitals have their own pharmacies for outpatients. Every two years, the Ministry of Health and Welfare (MHW) sets 'official' prices for all drugs, and these prices are supposed to determine the charges to patients and the national health insurance system. But pharmaceutical companies, as a matter of standard marketing strategy, offer the drugs to hospitals at a discount. The permitted discount is 10 per cent, but currently discounts are typically 20–30 per cent or more, particularly when several companies are competing for a share of the market. There is thus a tremendous financial incentive for doctors to prescribe drugs excessively and unnecessarily because they make a profit on every sale. Those new to Japan are often surprised when they go to a doctor with a simple cold and are given an injection of vitamin B and are loaded with drugs to take away. Another odd feature is that drugs are unlabelled, so patients do not know what they are taking.

TABLE 1 Sales of pharmaceuticals for human consumption, 1987

Country	Sales ($ × 10^9)	Expediture per capita ($)
United States	28.965	109
Japan	22.698	166
West Germany	7.606	136
France	6.754	128
Italy	5.962	109
Britain	2.596	51
Spain	2.053	51
Netherlands	0.964	57
Belgium	0.870	114

Source: DIALOG PTS PROMT.

Apart from being a massive drain on the national health system and on the pockets of patients, over-prescription and over-use of drugs has had some disastrous consequences. A classic example is that of blood products. In the early 1980s Japanese pharmaceutical companies began to import large amounts of cheap blood products from the United States, selling them to hospitals and doctors at well below the official price. By 1985 annual per capita blood-product consumption in Japan had risen to about three times that of Britain and France[1]. Coincident with the surge in imports, AIDS was introduced to Japan in non-heat-treated blood coagulants used for the treatment of haemophiliacs. Japan has about 1,000 carriers of AIDS, more than 90 per cent of whom are haemophiliacs.

Other examples are the anti-cancer drugs PS-K (Krestin), OK-432 (Picibanil) and Tegafur, all available only in Japan. Research on Tegafur has been abandoned elsewhere in the world[2], but in Japan Tegafur, together with Tegafur uracil, has annual sales of $280 million (Table 2). PS-K and OK-432 are widely prescribed — PS-K for cancers of the digestive organs (stomach, oesophagus, colon and rectum), lung and breast; OK-432 for cancers of digestive organs (stomach, liver, biliary tract, colon and rectum), head, neck, thyroid and lung. Total sales of these and other anti-cancer drugs peculiar to Japan are about $1,000 million per year. But have these enormous expenditures resulted in an increased number of lives saved? The simple answer is probably no.

Since the mid-1960s, survival rates for various cancers have steadily improved, but comparison of the rates with those of other countries where these drugs are not available[3] shows no evidence of increased survival in Japan (Table 4). The exception

TABLE 2 Top-selling anti-cancer drugs in Japan and the United States, 1987

Japan				United States		
	Drug	Sales ($¥ \times 10^8$)*	Percentage of market share	Drug	Sales ($\$ \times 10^6$)	Percentage of market share
1.	PS-K[†§]	515	25.2	Doxorubicin	86	16.3
2.	OK-432[†§]	275	13.4	Cisplatin	79	15.0
3.	Tegafur uracil[§]	255	12.5	Tamoxifen citrate	68	12.9
4.	5-Fluorouracil	150	7.3	Etoposide	52	9.8
5.	Tegafur[‡§]	145	7.1	Cyclophosphamide	31	5.9
6.	Tamoxifen citrate	90	4.4	Methotrexate	29	5.5
7.	Interferon–β	58	2.8	Megestrol acetate	27	5.1
8.	Lentinan[†§]	45	2.2	Mitomycin C	25	4.7
9.	Carmofur[§]	38	1.9	Bleomycin	20	3.8
10.	Estramustine phosphate sodium	36	1.8	Vincristine sulphate	18	3.4

Source: Estimate of Pharmaceutical Industries.

[*] ¥144 = $1. [†] Kinds of biological response modifiers.

[‡] Listed as an investigational drug in *AMA Drug Evaluation*, 6th edn (Saunders, Philadelphia, 1986).

[§] There is no entry for these drugs in standard manuals, meaning they are not recognized drugs in the developed nations.

is stomach cancer, but the higher survival rate of these patients is most likely to be due to early diagnosis in Japan.

TABLE 3 Documented adverse reactions to drugs sold only in Japan

Date disclosed	Drug	Date of approval	Side effects	Victims (deaths)
Sep. 1987	Peplomycin	Mar. 81	Interstitial pneumonia	? (8)
Jul. 1988	Carmofur	Sep. 81	Leukoencepharopathy	44(?)
Dec. 1988	Aspirin DL-lysine	Jun. 82	Shock	26(5)
Feb. 1989	Calcium hopatenate	Nov. 83	Severe encepharopathy	47(11)
Jun. 1989	Medroxyprogesterone acetate, high dose*	May 87	Myocardial, brain infarction	33(11)
Aug, 1989	OK-432	Oct. 75	Delayed type shock	13(3)

*This drug is sold in low doses in the United States and Europe.

TABLE 4 Comparison of five-year relative survival rates*

Sex and site of cancer	Osaka 1978–80	United States 1973–79		Britain 1971–73	Finland 1972–75
		White	Black		
Male					
Stomach	36.4	12	13	7.4	9.5
Colon	39.2	47	41	29.6	32.3
Lung	9.8	10	8	7.8	7.4
Female					
Stomach	28.4	14	16	7.3	8.3
Colon	31.0	49	47	29.4	31.2
Lung	9.6	14	11	7.0	9.8
Breast	69.1	72	60	56.8	57.6

Source: ref. 3. *Figures are percentages.

PS-K is of very doubtful efficacy. Studies during clinical trials seem to show some evidence of increased survival among cancer patients[4-6], but most of the research was published in company-financed or company-related journals; the only report in an international journal is a review of the Japanese work[7]. Although comparative studies were carried out in these clinical trials, they were severely flawed. For phase 3 studies, for example, disqualifications are a source of potential bias[8]. In the trials the disqualification rates for major end-points were 18 to 36 per cent, greater than the magnitude of the differences in outcomes tested. Furthermore, there was no assessment of whether patients complied with the requirements of the protocol, no description of procedures to ensure that the data are reliable and no analysis of whether there were significant deviations in findings from one institution to another. Also, the results have not yet been reproduced.

The MHW has reinvestigated the efficacy of OK-432 and PS-K, and a decision to restrict their use to combinations with other chemotherapeutic agents is imminent, according to Japanese newspaper reports. But as the drugs are usually used in combination with other agents, this is unlikely to have much effect on prescriptions and sales.

So why is PS-K so widely used despite its questionable efficacy? One reason is that it has no side effects. In Japan, doctors tend not to tell their patients if they have cancer, reasoning that the shock might kill them. PS-K is thus a handy drug to prescribe as it is taken orally and has none of the tell-tale side effects (such as hair loss) of anti-cancer drugs used in the West. Another reason is that most Japanese doctors are unfamiliar with the discipline of medical oncology. Finally, once a drug has passed clinical trials, the MHW tends to approve it for a wide range of related diseases without assessing its therapeutic efficacy or clinical usefulness for each disease. MHW has approved the use of interferon-α for kidney cancer and multiple melamona, and that of interferon-β for glioma and melanoma, for example. But although interferon can produce some response in tumours of these cancers, at present there is no evidence of an increased survival rate among patients. In the United States, the FDA has approved interferon-α only for hairy-cell leukaemia and melanoma.

To understand why there are so many dubious drugs on the Japanese market, one has to understand the unhealthily close relationship that exists between doctors, pharmaceutical companies and the MHW. Data presented to the MHW for approval are in mainly in the form of unpublished results or of reports published in Japanese in company-financed or company-related journals. Those in charge of clinical trials often set up their own foundations, funded by the pharmaceutical companies, for which they organize clinical trials. And often some heads of clinical trials also belong to the drug-approval committee. Thus a data-producing mechanism by the company for the company is inherent in the system.

What can be done? As some first steps I suggest the following.

- Only data published in leading international journals should be acceptable, and the work of more than one group of researchers must be available for evaluation; claims for drug effectiveness appearing in Japanese journals (except scientific society journals) should be discounted, as should unpublished results.
- The published evidence should be checked by referring to the individual patient's chart, and the data on the drug's toxicity and adverse effects should be examined.
- The MHW staff must be able to judge whether or not the papers are scientifically sound. The diagnostic criterion concerned must be checked and the results strictly interpreted according to the backgrounds of the patients concerned.
- In arriving at a decision, MHW staff must carefully analyse the risk-to-benefit, cost-to-benefit and cost-to-effectiveness factors, and should address questions such as what sort of benefits the drug will have. Needless to say, the members of the approval committee and MHW staff must be scientifically qualified (at present many are not). MHW should also enforce "good clinical practice" (ref. 9) on clinical trials, direct doctors to obtain informed consent properly, encourage them to pursue well-designed and controlled phase 3 studies, and assess adverse drug reactions.
- To cut down on Japan's wasteful consumption of drugs, pharmacies run by hospitals and individual doctors should be abolished and replaced with independent outlets. MHW is moving in this direction (a process called *bungyo*, meaning divide) but very little progress has been made.

Many of the problems I have described stem from the poor quality of science practised by Japanese clinicians, which in turn stems from the inability of most of them to read the foreign literature. But the problems are more deep-rooted than just language. In Japan there is little appreciation of the value of individual thought or philosophy which sustains science and is the spring of new ideas elsewhere — the lack of informed consent in medical practice is just one manifestation of that failing.

It seems that things have not changed much since 1902, when Dr Rintaro Mori (Ougai Mori), pointed out the Japanese were unable to sustain science but were very enthusiastic about eating its fruits. This attitude partly explains why Japanese research tends to be mostly method-orientated and unoriginal, and why some scientific fields created by other developed nations — clinical pharmacology and medical oncology, for example — are not established here. Radical, structural changes are needed if Japan's drug-approval system is to work for the benefit of the public and not for that of the pharmaceutical companies. ☐

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1. Swinbanks, D. *Nature* **332**, 193 (1988).
2. Friedman, M.A. & Ignoffo, R.J. *Cancer Treat. Rev.* **7**, 205–213 (1980).
3. Suemosu, K. (ed.) *Figures on Cancer in Japan, 1987* (Foundation for Promotion of Cancer Research, National Cancer Centre, Tokyo, 1987).
3. Friedman, M.A. & Ignoffo, R.J. *Cancer Treat. Rev.* **7**, 205–213 (1980).
4. Nakao, I. *et al. Oncologia* **14**, 163–170 (1985) (in Japanese).
5. Nakajima, T. *et al. Jap. J. Cancer Chemother.* **12**, 1850–1863 (1985) (in Japanese).
6. Niimoto, M. *et al. Oncologia* **14**, 171–180 (1985) (in Japanese).
7. Tsukagoshi, S. *et al. Cancer Treat. Rev.* **11**, 131–135 (1984).
8. Simon, R. & Wittes, R.E. *Cancer Treat. Rev.* **69**, 1–3 (1985).
9. *Standards for Clinical Trials of Drugs: Notice of the Pharmaceutical Affairs Bureau No. 874* (Pharmaceutical Affairs Bureau, MHW, Tokyo, 1989).

Reflections on the discovery of fission

Rudolf Peierls

Sir Rudolf Peierls adds a personal postscript to a year that marked the fiftieth anniversary of a discovery that helped shape the twentieth century.

THE publication of the paper by Hahn and Strassmann[1] in *Naturwissenschaften* in February 1939, 50 years ago, is rightly considered the essential step in the discovery of fission, although it was preceded by several years of experiments. But the background to this discovery lies much further back.

In 1932, the neutron was discovered by James Chadwick. It was immediately clear that it was a constituent of atomic nuclei, and soon it also became clear that nuclei consist of only neutrons and protons; there are no electrons in nuclei. The electrons emitted in beta decay are created in the act of emission. This insight marked the beginning of nuclear physics as we know it today.

Enrico Fermi in Rome was one of the first to realize that neutrons could become an important tool for studying nuclei. He became excited about this prospect and, although he had previously done important work in theoretical physics, he decided in 1933 to become an experimenter. By 1934 he had built his equipment and started experimental work with a group of collaborators.

The only way to generate neutron beams then was to bombard light elements with charged particles, either alpha particles of the kinds emitted from radioactive sources or protons from an accelerator, such as a cyclotron or a high-voltage installation. Rome had no accelerator, so Fermi was compelled to use the laborious method of directing alpha particles at beryllium.

Early observations

Fermi discovered that, in many elements, slow neutrons are more efficient at hitting nuclei than fast neutrons, which makes the experiment easier, provided one interposes between source and target a subtance containing hydrogen or other light elements, a 'moderator', in which the neutrons can collide with the light nuclei and lose kinetic energy to them.

Even those early observations were a puzzle for the then rudimentary theory of nuclei. It was then generally assumed that one could treat the passage of a neutron through a nucleus in good approximation as if it were moving in a potential field, which would be the average field of the particles inside the target nucleus. This picture, taken over from atomic theory, would of course have to be corrected for exchange of energy between the neutron and particular target nucleons, but this was taken to be a small correction.

On this basis, one would have expected the main result of a neutron–nucleus collision to be elastic scattering of the neutron, with an occasional inelastic event leading to excitation of the nucleus, and only very rarely the capture of the neutron with the emission of radiation. But the experiments showed that neutron capture was a very frequent event — for many nuclei, much more probable than scattering.

These findings completely mystified theoretical physicists until the solution was presented[2] by Bohr in 1936. Briefly, his point was that the constituents of the nucleus are interacting very strongly with each other, and with the incident particle, so that its kinetic energy is shared between many particles, with the result that none of them can escape until enough energy happens to be concentrated on one of them. This means that the neutron must remain in the nucleus for a much longer time than the picture of a single particle in a potential would have predicted and that, during that time, the chance of the emission of a photon is appreciable. In the case of a slow neutron, for which practically the whole excess energy is required for escape, escape becomes a very infrequent event and the emission of radiation dominates.

In most elements, the capture of a neutron produces a nucleus which is beta radioactive. (Artificial beta radioactivity had been discovered by Fréderic and Irène Joliot-Curie in 1933.) For different radioactive nuclei produced in this way, the beta rays have different half-lives, so that it is possible to distinguish the radioactive species formed. By chemical separation of the activated target, one can discover the chemical nature of the radioactive nuclei produced.

After establishing the principles, Fermi and his group bombarded with neutrons all elements they could get hold of, practically the whole periodic system of elements. This exploration produced a large amount of data, from which one could learn much about the properties of nuclei. A particularly interesting case was uranium, because that was the heaviest nucleus and at the end of the periodic system. If uranium captured a neutron it would become heavier. Because the number of neutrons in it would then be too large for the number of protons, it would probably increase its charge by emitting a beta electron and would thus become a new element, not existing in nature — a 'transuranic' element. The experiments on uranium were therefore watched with particular interest.

But the results were very unexpected. Instead of one, or a few, half-lives, the decay curves showed there were several, so many that it was not easy to disentangle the curves and find how many there were. How could neutron capture produce so many different species of nuclei from one element? This was a puzzle that many physicists tried to solve. Could a nucleus be found in many different states, without quickly being transformed to the most stable of them? Examples of such 'isomeric states' were known, though not in such large numbers. Niels Bohr was one of the theoreticians who tried to invoke such isomeric states to explain the behaviour of the alleged 'transuranic elements'.

Some answers

A different answer was proposed by a chemist, Ida Noddack-Tacke, as early as 1934[3]. She started from the fact that in his attempt to identify the chemical nature of the active nuclei, Fermi had looked only at elements of atomic number Z above 82, because none of the reactions known or imagined in nuclear physics could have resulted in a lower Z. Noddack-Tacke suggested that perhaps the uranium nucleus could have split into several sizeable pieces, whose atomic number then would be much smaller. So the experimenters should therefore look for activities following the chemical behaviour of much lighter atoms.

Nobody seems to have taken this suggestion seriously. Fermi (to whom the author presumably sent a copy of her paper) said such a capture of the neutron would not be sufficient to overcome the forces holding the nucleus together. Probably others who saw the paper also had difficulty in accepting the idea of a reaction that was so different from what had ever been seen.

Efforts to resolve the puzzle continued for many years. In Berlin, Hahn, Lise Meitner and Strassmann did painstaking chemical analyses to identify the chemical properties of the radioactive nuclei created. Joliot-Curie and Pavel Savich analysed one particular activity very carefully and found that it appeared to belong to an element with chemical properties indistinguishable from lanthanum, which saw the situation more or less as Landau saw it, but nobody published such speculations.

So it was natural that after the discovery of fission the question arose whether the fission was accompanied by the emission of secondary neutrons. The first positive answer to this question was reported by Joliot's group in Paris[11]. Their estimate of the number of neutrons per fission was somewhat high. More accurate measurements were done a little later by Fermi's group at Columbia University, New York[12].

A chain reaction requires that, of the neutrons released in the fission of one nucleus, more than one neutron should cause the fission of another nucleus. This requires that the loss of neutrons by other processes be below a certain limit; if this condition is met, there will be a critical size, because in too small an amount of material, the proportion of neutrons leaving the body before hitting a uranium nucleus would be too large.

By now, the possibility of a chain reaction had become more real, but there were still difficulties. As Bohr had pointed out, the abundant isotope ^{238}U can be split only by fast neutrons with energy above a threshold now known to be about 1 MeV. Only a fraction of the neutrons created in the fission process have such high energies, and they tend to lose energy by inelastic collisions.

The large effect of slow neutrons on ^{235}U suggested slowing down the fission neutrons by adding to the uranium a moderator, that is, a light element. The neutrons collide with the atoms of the moderator and thereby lose energy. But the moderator also absorbs some neutrons, and for most light elements the absorption is great enough to reduce the average number of neutrons per fission to less than unity, thus making a chain reaction impossible. Moderators that have been used successfully with natural uranium are carbon, in the form of graphite, and heavy water (D_2O).

Fermi succeeded in making the first chain reaction by using a graphite moderator. For this, commercially pure graphite would not do because certain impurities can absorb too many neutrons even if they are present in concentrations undetectable by chemical means. Fermi insisted on further purification, in the belief that there were still excessive impurities, and indeed the absorption of neutrons was reduced below the critical level. With this purified graphite the first chain reaction was produced by Fermi's group in Chicago on 2 December 1942.

The success of this experiment opened the way to the release of nuclear energy on a macroscopic scale. The most obvious application was to power generation. This would consist of reactors working in principle like Fermi's first experimental one, but with arrangements for heat transfer, which allowed the heat generated in the uranium to be passed to steam generators or other devices for driving machinery. There were of course many practical problems to be overcome, but they were technological rather than nuclear physics problems.

Another application was to the production of radioactive isotopes, which are useful in many areas of research, and for medical diagnosis and treatment. Nuclear reactors are an abundant source of neutrons, which can be used to bombard various targets and to produce in them a great variety of radioactive species. But at the time all these possibilities were for the future. The world was at war, and interest was concentrated on the third possible application, the nuclear bomb.

Bohr had pointed out that a reactor could not produce a violent explosion. The reactor depends on slow neutrons, which have to travel considerable distances before they cause further fission, so that the development of the chain reaction is slow. When the released energy has heated the reactor to a temperature that will melt or vaporize its materials, the expansion and dispersal will stop the chain reaction. The energy released is therefore only of the order of the cohesive energy of the structure, so that the explosion is no more violent than that from chemical explosives.

In 1940, I was discussing such problems in Birmingham with Frisch, and we felt rather reassured by Bohr's argument, which seemed to show that a fission bomb was impossible. Then, one day, Frisch asked me: "Suppose someone gave you a lot of separated ^{235}U, what then?" This seemed an entirely academic question because the separation of isotopes had been achieved only in gram quantities and only for light elements, where the fractional mass difference of the isotopes is much greater than the roughly 1 difference in uranium.

With ^{235}U, every neutron is capable of causing fission, and one does not need to rely on the especially high capture rate for slow neutrons, so one needs no moderator. We could guess at the nuclear cross-sections from Bohr's theory, and I had a formula ready to give the critical size in terms of the cross-sections. We were staggered to find that the critical size, or mass, was only pounds, instead of the tons one intuitively expected after thinking about reactors.

How much energy would be released in such a fast chain reaction? Now the neutrons were 10,000 times faster than the thermal neutrons effective in the reactor, and they had a much shorter distance to travel. Therefore the build-up of the chain reaction would be fast enough to cover an appreciable fraction of the uranium nuclei before the pressure from the heat developed would disperse the materials and stop the reaction. One would have an explosion of unprecedented power.

It seemed to us that the enormous power of such a weapon would be worth the great effort required to separate the uranium isotopes. This was in 1942, and we feared that Nazi Germany might complete such a weapon first. We knew there was no defence except the threat of retaliation.

We wrote a memorandum to the British authorities, in which we presented our arguments and outlined, as best we could, the implications. We mentioned the fallout and the inevitability of many civilian casualties in almost any use of such a weapon. We did not foresee the fusion bomb, nor the insane arms race.

These arguments were taken seriously by the British government, and this in turn led to an accelerated pace in setting up a large-scale project in the United States — the Manhattan Project. This succeeded in separating large amounts of ^{235}U, but also followed an alternative approach: some of the ^{238}U nuclei capture a neutron to produce ^{239}U which is itself unstable and emits a beta electron to become neptunium-239 (^{239}Np). That, in turn, undergoes rapid beta decay to become plutonium-239 (^{239}Pu), which lives for a long time. It was conjectured from Bohr's theory, and later verified, that in ^{239}Pu, as in ^{239}U, neutrons of any energy would cause fission; it was also weapons material. So another activity of the Manhattan Project was to build large reactors producing plutonium. It is common knowledge that both these methods led to the production of nuclear weapons and that they were used against Japan.

This is not the place to discuss the moral implications of the making and the use of the bomb, or the best way of avoiding nuclear war, or to argue the advantages and dangers of nuclear power. My objective has been to tell the story of the very esoteric work of academic physicists and how in such a short time it developed into applications of enormous military, political and economic importance. ☐

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1. Hahn, O. & Strassman, F. *Naturwissenschaften* **27**, 89 (1939).
2. Bohr, N. *Nature* **137**, 344–348 (1936).
3. Noddack-Tacke, I. & Angewandte, Z. *Chemie* **47**, 653 (1934).
4. Joliot-Curie, I. & Savit, P. *C. r. hebd. Séanc. Acad. Sci., Paris* **206**, 1643 (1938).
5. Hahn, O. & Strassman, F. *Naturwissenschaften* **27**, 11 (1939).
6. Meitner, L. & Frisch, O.R. *Nature* **143**, 269 (1939).
7. Frisch, O.R. *Nature* **143**, 276 (1939).
8. Bohr, N. *Phys. Rev.* **55**, 418 (1939).
9. Nier, A.O. *et al. Phys. Rev.* **57**, 546 (1940).
10. Bohr, N. & Wheeler, J.A. *Phys. Rev.* **56**, 426 (1939).
11. Halban, H.V., Joliot, F. & Kowarski, L. *Nature* **143**, 470 (1939).
12. Anderson, H.L. & Fermi, E. *Phys. Rev.* **56**, 284 (1939).

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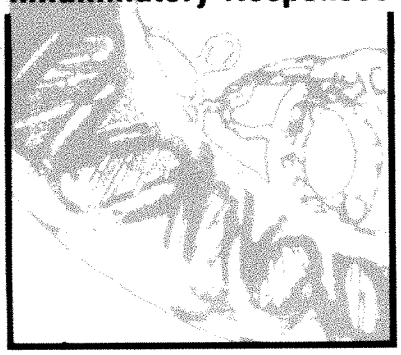

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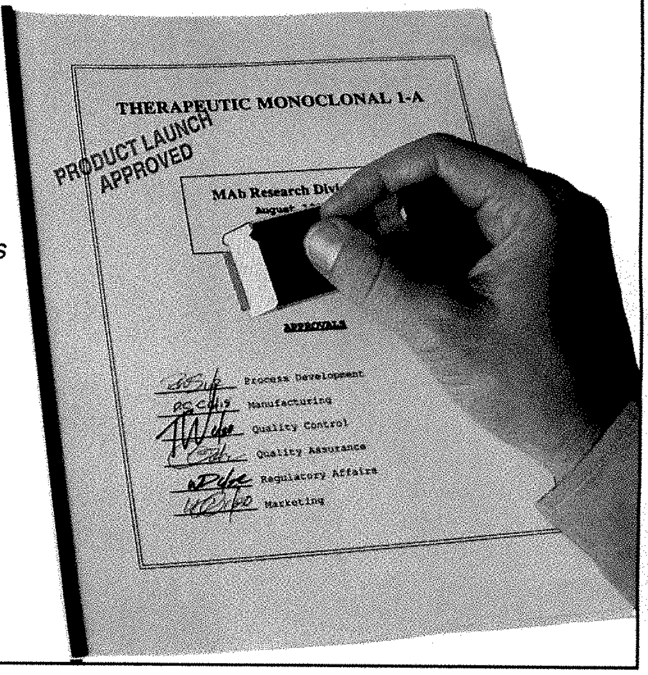

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Making authors toe the line

The idea that those who wish to contribute to the scientific literature should first satisfy preconditions sends shivers down the spine: are there compromises to be worked out?

DATABASES have come to stay and, like many other institutional innovations, have roused great passions. So much is plain from the manner in which the supporters of particular databanks advocate their virtues — with zeal, sometimes with blind zeal. Only a few weeks ago, in a nice illustration of that genre, one correspondent found himself declaring that "it is a simple matter to require that the author of a sequence paper provides an accession number . . . as a prerequisite to publication" (*Nature* **342**, 114; 1989). But is it?

So far as this journal is concerned, most passion centres on the decision that preconditions on the publication of research material are not simple. In particular, it has not hitherto been a precondition of the consideration of a manuscript for publication that nucleotide sequence data (if any) should first have been registered with one of the three branches (in Europe, the United States and Japan) of the international enterprise for compiling a coordinated nucleotide sequence databank. What follows is yet another explanation of what is plainly a contentious issue and of what some may even consider to be a remedy, this time evoked by the letter from Dr Lennart Philipson and his colleagues at Heidelberg (see page 849).

The first need, obscured in many of the arguments conducted in recent months, is that we should acknowledge the virtues of most databases, and of the nucleotide sequence databank in particular. It would be a great public service if there were a generally and uniformly available set of nucleotide sequences to which researchers could refer. Despite operational difficulties (many of them teething troubles) at the three centres, the service they provide is a good first approximation to a uniformly accessible sequence databank. There is good reason to expect that the arrangements now in place will be able to handle the much increased volume of data expected in the next few years. So far, it might be thought, so good. These are some of the reasons why this journal has, for several years, "urged" its authors to send their data to the sequence databanks. So why not go the whole hog, and make that a precondition?

This is not the arcane argument it may seem, but rather an issue of principle touching the relations between journals such as this and their contributors on the one hand and their readers on the other. It is not, of course, absurd, or an infringement of contributors' rights, that journals should require their contributors to satisfy certain preconditions — so many copies of a manuscript, typed in double-spacing, for example. Their justification is the mutual convenience of journals and their contributors that decisions should be reached quickly and that manuscripts chosen for publication should be as quickly processed. Tacitly, at least, such points are easily agreed.

Difficulties arise when contributors are asked to satisfy conditions that have nothing to do with the content of what they have to say, or the manner in which that is presented. Compliance with the wishes of the databanks is only one of the requirements that might be made of them. Why should not journals also demand, for example, that their contributors should have complied with the law? Would it not make for the tidier administration of regulations on the use of recombinant DNA techniques that the appropriate committees, if relevant, should have been notified and have given their approval? And the same, *mutatis mutandis*, for embryo research, the use of radioactive chemicals in the laboratory or the use of experimental animals in research? While they are at it, why should not journals also take on the administration of national legislation on secrecy?

Not all those who urge this journal to make mandatory the prior submission to databanks of nucleotide sequences would go so far as to applaud all possible innovations along these lines. An earlier correspondent (*Nature* **342**, 114; 1989) correctly argued that the interaction between journals and their contributors, usually anxious to appear in print, is a point at which pressure can be effectively applied. But that is all the more reason why journals, rightly fearful of being turned into instruments of law-enforcement, should resist it. And the only simple recipe for the avoidance of the consequences of prior conditions on publication is that there should be none.

Relations between journals and their readers are also important. The business of publication, which is an honourable and socially valuable business, is that of making information generally available. In principle, at least, all readers should be on an equal footing, with an equal chance to make what they can of what happens to be published. That is why this journal has, uniquely among journals with an interest in research, taken the trouble to print itself at three centres in world (so far). It remains a constant source of disappointment that the West Coast of the United States and Australia should be less quickly served than, say, New York or London.

Readers' appreciation of what they read depends, of course, on circumstances. People somehow in the swim are usually better placed to appreciate the full significance of what they find it interesting to read than those exiled to the sticks. Often it may be that a reader's understanding of a novel concept in a research article will fully flower only when he has talked it over with his or her colleagues, which is another kind of privilege. Most journals prudently acknowledge that these are circumstances they cannot change, much as they might wish to do so. But there are no circumstances in which a journal such as this, founded 120 years ago to bring the record of research to a wide audience, could fall in with the idea that its readers' appreciation of what they read will hang crucially on the accessibility of a databank in Heidelberg, Los Alamos or Mishima.

Accordingly, this journal has determined as follows: we shall continue (as at present) not to require of our contributors that they should have submitted their data (if in any way relevant) to the international databanks before submitting them for publication, but we shall threaten them that, if their articles appear in print and happen to be supported by nucleotide sequences, even if not intended to be published, we shall send those data off to Heidelberg or whichever other centre appears more congenial and also promises to make the data more generally available. We shall also make exactly the same data available to such of our readers who happen to telephone or to write. We shall do that without rancour, but shall aim to give the databanks a good run for their money. From time to time, we shall be willing to listen to people who have special secrets to hide.

This, then, is this journal's proposition: people with a nucleotide sequence to disclose should either disclose it or keep mum. They should not seek the best of both worlds, that in which they get credit for the sequence without saying what it is. Nor, for that matter, should they expect to be able to publish a sequence of nucleotides without being able to guess at what it means. Somewhere between, there must be a happy mean. Curiously, that boils down to an editorial function, not an issue of principle.

John Maddox

PLATE TECTONICS

Helium line in the Banda arc

Eli A. Silver

A LONG-STANDING enigma in geotectonics is just what melts to produce arc magmas in a subduction zone — where the cold, dense edge of oceanic lithosphere (crust and upper mantle) sinks into the deeper mantle. Can one recognize in the arc lavas differences in material entering the subduction zone? One approach to these questions is to use geochemical markers in the volcanics of island arcs, and a number of such techniques are known[1]. Results reported by Hilton and Craig on page 906 of this issue[2] show that helium isotope ratios, a useful geochemical marker, in island-arc basalts achieve a spatial resolution unequalled by other studies. Simultaneously, they identify remarkable processes occurring at the Sunda–Banda volcanic arc, a subduction zone in the Pacific ocean.

Earlier this year, Poreda and Craig[3] reported on a survey of circum-Pacific island arc studies of the ratios (R) of ^{3}He/^{4}He in arc materials relative to those (R_a) in air. (^{4}He is radiogenic and must be derived from contamination by continental crust.) They discovered that most of the circum-Pacific arcs have ratios R/R_a close to that expected from mid-ocean ridge basalts (MORBs), indicating that it is the mantle wedge above the slab, rather than the slab itself, that melts. The significant exception to this observation is at the Banda arc, where the ratios are much lower, indicating contamination from the subducting Australian continent. It is the data from the Sunda and Banda arcs that Hilton and Craig[2] now examine more closely. The authors demonstrate that the change from higher (MORB) ratios to lower (contaminated) values occurs over a narrow (200 km) zone. This transition corresponds with a well-defined zone of seismicity, which they interpret as faulting between the subducting Indian ocean and Australian continental lithospheres[4] (Fig. 2).

Gross differences in gravity, morphology, seismicity and volcanism between the Sunda and Banda arcs have long been known[5]. These differences have been best explained by the change in subduction from oceanic to continental crust. Hypothetical transform faults have been proposed to explain some surface geological variations, but these have not been substantiated by close scrutiny. The seismological studies[4] were the first to indicate a narrow zone of fault activity within the lower plate.

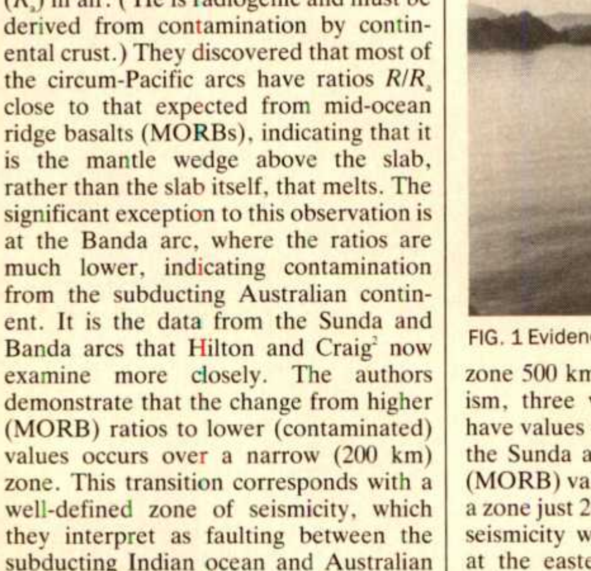

FIG. 2 Schematic diagram of transition between oceanic and continental lithosphere in Australian plate subducting beneath the Banda arc. Note source of arc magma in mantle wedge, generally about 100 km below the surface.

Hilton and Craig find that R/R_a ratios in the eastern Sunda arc, which includes Java, Bali, Sumbawa and western Flores, exceed those on eastern Flores and Pantar by factors of 2–4. Passing east of a zone 500 km wide without active volcanism, three volcanoes of the Banda arc have values 4–8 times lower than those of the Sunda arc. The transition from high (MORB) values to low values occurs over a zone just 200 km wide. The zone of high seismicity within the lower plate occurs at the eastern end of this geochemical transition.

Frank Lane Picture Agency

FIG. 1 Evidence of activity: Banda volcano on the eastern section of the Sunda – Banda arc.

It is reasonable to conclude from these studies that it is oceanic lithosphere that underlies the Sunda arc west of central Flores, and that contamination of the mantle wedge begins beneath eastern Flores. This initial zone of contamination is west of the zone of intense seismicity in the lower plate, so the latter may represent the transition from oceanic to continental lithosphere. The contaminants seen geochemically by Hilton and Craig could then indicate melting of continental margin sedimentary rocks, rather than the crustal rocks themselves.

But why the sharp transition? If the contamination is by sediments, would not the effect be gradual? The answer may lie in the structure of the western edge of the Australian continent. South-east of Sumba island, the lower plate has a fracture zone (an ancient transform fault) that divides the trench over a very narrow zone, about 25 km wide[6]. The fracture zone trends north-east, and its extension would place it very near the transition zone mapped geochemically by Hilton and Craig. East of the fracture zone, sediments several kilometres thick are entering the trench and a wide accretionary wedge is formed by scraping off the subducting trench sediments and accreting them onto the fore arc. West of the fracture the trench is nearly empty and the trench slope is steep, suggesting little accretion. If the surface structure shows so sharp a transition, might not the deeper mantle wedge be similarly affected? The results of Hilton and Craig imply that it is. They also show an antithetic relation between ^{87}Sr/^{86}Sr and R/R_a for the Sunda arc, but not for the Banda arc. This relationship supports the idea that two-component mixing of He and Sr is occurring over a large segment of the plate boundary, and has not been previously documented.

One might ask whether the Sunda–Banda arc structure is unique, or are there other examples? Poreda and Craig[3] measured somewhat lower values of R/R_a for Taiwan, a well-documented zone of subduction of continental crust. The ratio is slightly reduced in the eastern Aleutians, the Kuriles and Kamchatka, and Japan, which may be explained in part by thick sediment input to these trenches. No reduction is seen, however, in the Cascades arc, whose trench is thickly sedimented. No He data are yet available for the New Britain arc and the volcanoes north of Papua New Guinea, which lies in a somewhat different collisional regime to the Banda arc. The oceanic lithosphere beneath the Banda arc is continuous with the continent, and drags the continental rocks with it[4], whereas in the Papua New Guinea collision the oceanic lithosphere appears structurally separated from the continent[7]. That difference may explain the unique results of Hilton and Craig in the Banda arc — and it can be tested. □

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1. Gill, J.B. *Orogenic Andesites and Plate Tectonics* (Springer, Berlin, 1981).
2. Hilton, D.R. & Craig, H. *Nature* **342**, 906 – 908 (1989).
3. Poreda, R. & Craig, H. *Nature* **338**, 473 – 478 (1989).
4. McCaffray, R., Molnar, P., Roecker, S.W. & Joyodiwiryo, Y.S. *J. geophys. Res.* **90**, 4511 – 5428 (1985).
5. Nishimura, S. *et al. Geol. Res. Devl. Centre Spec. Pub.* **2**, 105 – 113 (1981).
6. Breen, N.A., Silver, E.A. & Hussong, D.M. *Geol. Soc. Am. Bull.* **97**, 1250 – 1261 (1986).
7. Cooper, P. & Taylor, B. *Tectonics* **6**, 53 – 67 (1987).

CELL BIOLOGY

Mitosis and membranes

Graham Warren

WHEN cells divide, the chromosomes segregate and all other cellular components are partitioned between the daughter cells[1]. For most membrane compartments in animal cells the mechanism involves wholesale break-up; the cell, in effect, homogenizes the membranes yet does so in a manner which permits reassembly of the organelles. The pieces disperse randomly throughout the mitotic cell cytoplasm so that partitioning becomes a stochastic process dependent on the ability of the mother cell to divide itself into two daughters of equal size[2]. The molecular mechanism whereby membrane compartments are broken down is obscure, but the report by Tuomikoski *et al.* on page 942 of this issue[3] may provide a clue to what happens.

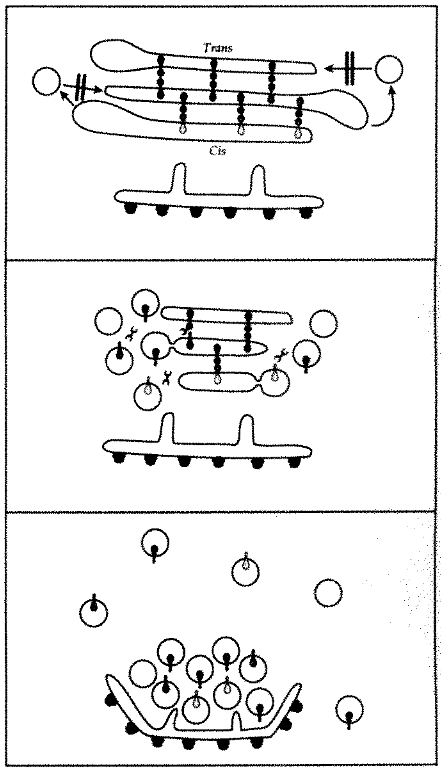
Morphological studies on organelle division go back to the beginning of this century[4], but progress was slow until the advent of electron microscopy and, more recently, immuno-electron microscopy. There are only single copies of the endoplasmic reticulum and Golgi apparatus in animal cells[5,6], so they have to be divided during mitosis. They are first broken into fragments and then vesiculated. The nuclear envelope always breaks down in animal cells, but the extent of vesiculation of the rest of the endoplasmic reticulum varies from cell to cell[7], perhaps because this organelle is present throughout the cell cytoplasm, and so does not need to be dispersed in order for partitioning to occur. In contrast, the Golgi apparatus, which has a restricted, juxtanuclear location[6], is completely vesiculated to ensure its dispersal and consequent partitioning[8].

Despite the apparent complexity of this process it is possible to suggest a very simple model for vesiculation[9] (see figure). The model demands separation of Golgi cisternae and cessation of vesicle fusion. Continued budding then converts the cisternae into free vesicles and clusters of vesicles which have been observed by immuno-electron microscopy[10,11]. Many lines of evidence point to an inhibition of membrane fusion during mitosis[9,12], but the evidence has, up to now, been indirect.

Tuomikoski *et al.*[3] have now shown that membrane fusion is inhibited in extracts from mitotic cells. Isolated endocytic vesicles were mixed in the presence of an energy source and frog cytosol, and fusion was shown to occur. When the cytosol was prepared from frog eggs arrested in metaphase II, fusion was inhibited. They also treated interphase cytosol with the sea-urchin homologue of $p34^{cdc2}$, the protein kinase of *cdc2*, which plays a key role in driving cells into the mitotic state[13]. This treated cytosol prevented fusion in a dose-dependent manner. Tuomikoski *et al.* have not, so far, shown that the effect of the extracts is reversible, but the absolute dependence of the reaction on $p34^{cdc2}$ is evidence in favour of their interpretation.

The link between endocytosis and intra-Golgi transport is provided by an *N*-ethylmaleimide-sensitive factor (NSF). This was known to be involved in the fusion of Golgi transport vesicles[14] and recent work has shown it to be involved in the fusion of endocytic vesicles[15]. Either NSF, or an associated protein of the fusion machinery, could therefore be the target for the inhibition during mitosis. But why inhibit the endocytic pathway? Is it simply the inevitable consequence of sharing the same fusion mechanism with an organelle that has to partition during mitosis? Or is it that endosomes are more elaborate and extensive than has so far been appreciated[16], and that they too have to be vesiculated and partitioned in the same way as the Golgi apparatus? It could be that most, if not all, intracellular compartments that communicate by way of vesicles are present in only one or a few copies and inhibition of membrane fusion provides the means of partitioning during mitosis.

The reason for having a single copy is not known, but it may be a device to unify the actions of an organelle. The resultant complex structures can then pose problems if the organelle needs to be moved to a new location in order to carry out a different function[17]. There is no reason why the partitioning mechanism should not work, during interphase, to facilitate such movement by way of vesicles. Mitosis would then represent the extreme when single-copy organelles are completely fragmented for partitioning.



Model for the vesiculation of the Golgi apparatus. Transported proteins move through the Golgi stack by way of vesicles which bud from the dilated rims and fuse with the next cisterna in the stack towards the *trans* side[20]. Transport occurs by bulk flow[21], which means that endogenous Golgi proteins such as the mannosidases and glycosyltransferases have to be prevented from entering the dilated rims. Lateral interactions might restrict their ability to act on glycoprotein substrates, so the simplest way to restrain them would be to link them to those proteins in the neighbouring cisterna which also need to be restrained. This would automatically generate the stack of cisternae which is the central feature of the Golgi apparatus. At the onset of mitosis transport vesicles would no longer be able to fuse (top panel) but budding would continue. At the same time, or shortly thereafter, the links between the cisternae would be broken allowing the endogenous Golgi proteins to move into the budding vesicles (middle panel). Continued budding would convert the cisternae into the vesicles (bottom panel) which are observed in metaphase animal cells[10]. Clusters of vesicles are also observed[11], though the material that binds them together has yet to be characterized.

Finally, one could ask why such a mechanism is used. Chromosomes, after all, use the mitotic spindle, so why is there not a more elaborate mechanism for segregating membrane-bound organelles? There are several possible reasons. First, unlike the chromosomes, there is no need to divide an organelle into two exactly equivalent pieces. If one daughter receives slightly more or less membrane, then mechanisms exist to correct the imbalance[18]. Second, the mechanism is straightforward in that it inhibits a pre-existing process, though additional modifications may be needed to ensure that the vesicles contain the information needed for reassembly[10]. Third, it is general because most, if not all, vesicle-mediated pathways share the same mechanism for fusing membranes, specificity being provided by other proteins[19]. Last, it is flexible. The vesicles generated are small and easily diffusible, so they should rapidly and randomly occupy the mitotic cell cytoplasm. This means that it does not matter what shape or size the cell is, so long as the cytokinetic mechanism divides it into two daughters of equal size[3]. The molecular mechanism underlying this membrane-partitioning process is of obvious interest and the report by Tuomikoski *et al.* is a step in the right direction. □

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1. Birky, C.W. *Int. Rev. Cytol.* **15**, 49–89 (1983).
2. Rappaport, R. *Int. Rev. Cytol.* **31**, 169–213 (1971).
3. Tuomikoski, T., Felix, M.-A., Dorée, M. & Gruenberg, J. *Nature* **342**, 942–945 (1989).
4. Wilson, E.B *The Cell in Development and Heredity* (Macmillan, New York, 1925).
5. Rambourg, A., Clermont, Y. & Hermo, L. *Meth. Cell Biol.* **23**, 155–166 (1981).
6. Louvard, D., Reggio, H. & Warren, G. *J. Cell Biol.* **92**, 92–107 (1982).
7. Zeligs, J.D. & Wollman, H. *J. Ultrastructure Res.* **66**, 53–77 (1979).
8. Lucocq, J.M. & Warren, G. *EMBO J.* **6**, 3239–3246 (1988).
9. Warren, G. *Trends biochem. Sci.* **10**, 439–443 (1985).
10. Lucocq, J.M., Berger, E.G. & Warren, G. *J. Cell Biol.* **109**, 463–474 (1989).
11. Lucocq, J.M. *et al. J. Cell Biol.* **104**, 865–874 (1987).
12. Beckers, C.J.M. & Balch, W.E. *J. Cell Biol.* **108**, 1245–1256 (1989).
13. Hayles, J. & Nurse, P. *Exp. Cell Res.* **184**, 273–286 (1989).
14. Malhotra, V. *et al. Cell* **54**, 221–227 (1988).
15. Diaz, R. *et al. Nature* **339**, 398–400 (1989).
16. Marsh, M. *et al. Proc. natn. Acad. Sci. U.S.A.* **83**, 2899–2903 (1986).
17. Trelstad, R.L. *J. Cell Biol.* **45**, 34–42 (1970).
18. Zorn, G.A., Lucas, J.J. & Kates, J.R. *Cell* **18**, 659–672 (1979).
19. Bourne, H.R. *Cell* **53**, 669–671 (1988).
20. Dunphy, W.G. & Rothman, J.E. *Cell* **42**, 13–21 (1985).
21. Wieland, F.T *et al. Cell* **50**, 289–300 (1987).

ECOLOGY

Answers that lie in the soil

Peter D. Moore

OUT of sight, out of mind, may be a convenient maxim for those with the inclinations of an ostrich. But it is hardly appropriate for ecologists concerned with estimating the productivity of the Earth's ecosystems, for much production occurs beneath the surface of the soil. The International Biological Programme (IBP) of the 1960s placed strong emphasis on the development of techniques for studying productivity and many data were accumulated which have subsequently been used in the assessment of the productive resources available for human support and, more recently, in developing a fuller picture of the Earth's carbon cycle. But many of these initial results are flawed by a failure to account adequately for the underground accumulation of carbon, and also for the continuous cycle of death and turnover of plant tissues above and below ground. Work by Raich and Nadelhoffer on forests[1] and by Long *et al.* on grasslands[2] now implies that some of the early results on this subject could be a serious under-estimate of the true values.

It would be wrong to suggest that the IBP pioneers were unaware of the problem of root productivity and turnover. Newbould[3] proposed that the ratio of below-ground biomass is proportional to that above ground and Newman[4] developed techniques for the measurement of the extent of plant roots within the soil by dissecting out sample soil volumes. Many field studies, however, neglected the subterranean part of the equation simply because of the difficulties involved in its measurement. Others estimated changes beneath the ground by subtracting the minimum from the maximum root biomass in a given year (Dahlman and Kucera in their studies of prairies[5], for example).

For forest ecosystems, there is considerable disagreement over the proportion of fixed carbon that is allocated to subterranean organs. Raich and Nadelhoffer[1] have collated and examined data derived from studies in a wide geographical range of forest ecosystems. They start by assuming steady-state conditions for their soil carbon cycle (which may itself be questionable) and derive an expression to show that the total carbon allocation to roots can be estimated by summing root detritus production and root respiration; this, in turn, is given by the soil respiration rate less the rate of above-ground litter production (which supplies soil heterotrophs with their additional source of carbon). Raich and Nadelhoffer conclude that there is a positive linear relationship between litter fall and carbon allocation to roots, but that, in proportional terms, relatively less of the fixed carbon is transported to roots in more productive forests. Ultimately, our knowledge of terrestrial carbon cycles will depend upon the precision with which such translocation can be predicted, and on more detailed evidence concerning the stability of the carbon reservoir in the soil.

Long *et al.*[2] present new results from a continuing UNEP study of four tropical grassland sites in Mexico, Kenya, Thailand and Brazil. They used 20 randomized sample plots, each 1.0m by 0.25m in size, which they cropped every month and determined the weight of living and dead tissues, both above and below ground. The root material was extracted from the soil cores using fine-mesh (2mm) sieves (no real advantage was found in using sieves of smaller aperture). They separated the live roots by staining with tetrazolium salts.

They were then able to calculate the turnover of plant matter, both above and below the soil surface, that is neglected in most standard procedures. In three of their sites they found that the traditional IBP methods had underestimated primary productivity by a factor of between two and five. On a global scale this means that there is substantially more productivity in the tropical grassland biome than has previously been realized.

These results have a bearing on both agricultural and carbon-cycling studies. In pastoralism, they imply that more energy is entering the ecosystem than had been thought. If a substantial amount of leaf material is dying and passing to detritivores, then one must conclude that the grassland is being under-used. Energy diverted to roots, however, is of no economic value to grazing animals. But even this aspect of carbon allocation has implications for global carbon-budget studies, for clearly there is a larger sink for carbon in the tropical grasslands than has previously been suspected, but only if some of the added carbon is accumulating in the soil. This does seem to be occurring in some of the grassland sites examined by Long *et al.* but much more effort needs to be expended on precise quantification of this build-up of organic matter. Equally important, the ploughing of grasslands for arable agriculture will inevitably result in the release of soil carbon back into the atmosphere. Perhaps the ostrich, with its head below the ground, was actually trying to tell us something. □

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1. Raich, J.W. & Nadelhoffer, K.J. *Ecology* **70**, 1346–1354 (1989).
2. Long, S.P. *et al. Plant and Soil* **115**, 155–166 (1989).
3. Newbould, P.J. *Methods for Estimating the Primary Production of Forests*, IBP Handbook No.2, (Blackwell Scientific, Oxford, 1967).
4. Newman, E.I. *J.appl.Ecol.* **3**, 139–146 (1966).
5. Dahlman, R.C. & Kucera, C.L. *Ecology* **46**, 84–89 (1965).
6. Singh, J.S., Lavenroth, W.K., Hunt, H.W. & Smith, D.M. *Ecology* **65**, 1760–1764 (1984).
7. Coombs, J., Hall, D.O., Long, S.P. & Scurlock, J.M.O. (eds) *Techniques in Bioproductivity and Photosynthesis* 2nd edn (Pergamon, Oxford, 1985).

SYNTHETIC CHEMISTRY

Chemists get topological

Roland Pease

MOLECULAR LEGO is the term Fraser Stoddart and colleagues have coined to describe their approach to chemical synthesis. Yet this almost understates the ease with which the reagents assemble themselves to form the catenane — a linked-ring compound — depicted below (Fig. 1). Their new work[1] builds on both the host–guest chemistry for which Pederson, Cram and Lehn gained the Nobel prize, and the notion of topological isomers developed by Frisch and Wasserman[2].

Catenanes are characterized by the linking together of molecular rings without the intervention of covalent bonding. The importance of the new work is in the exploitation of non-covalent 'donor–acceptor' interactions that make the reagents act as templates for one another. In this way, the authors obtain a 70 per cent yield of the catenane product from a simple one-step reaction (stir reagents 2,3 and 4 — Fig. 2 — together in a solvent and leave to stand overnight); and that yield represents merely the red precipitate deposited and does not take into account any product still in solution. By comparison, other synthetic routes are both more complex and less efficient.

The key to the authors' success was the recognition of the non-covalent forces at work in host–guest complexes. They noted that the roles of electron donor (red) and acceptor (blue) are interchangeable in binding the supramolecular complexes 5 and 6 (Fig. 3). This reciprocity, they argued, could be used to stabilize a reaction intermediate in which an electron-donor guest ring (compound 4) would hold, vice-like, one limb of the u-shaped acceptor compound 2. Effectively, the two rings of the catenane — one initially incomplete — act mutually as templates for the reaction (Fig. 2), which is completed by the ring closure by moiety 3. The ingenuity deployed in the new work, the culmination of ten years of systematic study, is characteristic of studies in 'topological' chemistry.

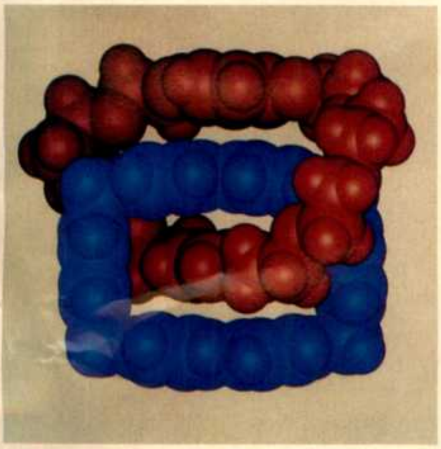


FIG. 1 Space-filling model of the [2] catenane (compound 1) synthesized by Stoddart and colleagues. Prepared on the basis of X-ray data by D.J. Williams and colleagues at Imperial College.

Previous attempts to synthesize catenanes have relied on the improbable threading of a large cyclic molecule onto a long single-strand molecule, on the remarkable topological properties of the Möbius strip, and on the ability of transition-metal ions to arrange organic ligand molecules in fixed orientations. The first method was also the first to work[3]. The two reagents are mixed in conditions that favour closure reactions of the linear molecule. In the rare event that ring closure occurs after the linear molecule has threaded the macrocycle, the product is [illegible] catenane that is topologically disti[illegible] from the main reaction product. Although only traces of catenane were made, their presence could be demonstrated by subtle use of deuterated reagents and analytical chemistry.

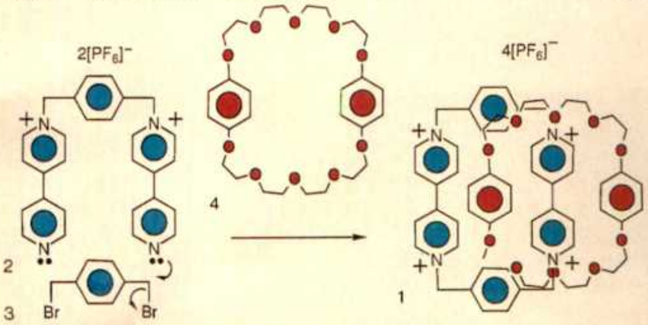


FIG. 2 Reaction scheme by which the [2] catenane (compound 1) is formed from a bispyridium salt $(2^{2+})\cdot 2PF^{2-}$, 1,4bis(bromomethyl)-benzene (3) and bisparaphenylene-34-crown-10 (4). The reaction was carried out at room temperature in dry acetonitrile (Blue, electron acceptor; red, electron donor.)

Cut a Möbius strip with two twists, and the result is a pair of rings with the same radius, but inextricably linked. The notion lends itself well to catenane synthesis, but the execution is not trivial. The problem is to find a double-stranded molecule with breakable crosslinks that is sufficiently flexible both to turn back on itself for ring closure, to form a loop and to twist twice on its axis. Nevertheless, a Möbius strip was successfully made (22 per cent yield) with a single twist in 1982 by D.M. Walba and colleagues[6]. The earlier attempt by Wasserman and colleagues[5,6] to synthesize the double-twist Möbius strip led only to trace quantities of catenane.

By far the most successful method to date is the use of transition-metal ions as templates for catenane synthesis. This approach, espoused by J.-P. Sauvage and colleagues[7], relies on the directionality of transition-metal–ligand bonds to dispose rigid reagent molecules in the correct arrangement for catenane synthesis. The ligands are either two half-rings or a half and a whole-ring which overlap and are completed by the addition of complementary half-rings. The final step in this synthetic route is to extract the strongly bound metal ion from the middle of the newly formed catenane without undoing the product. It turns out that the powerful ligand cyanide (CN^-) works admirably and yields of 50 per cent with respect to the starting materials are common. Not only have Sauvage and colleagues succeeded in making [2] catenanes (two interlocking rings) by this method, but also [3] catenanes and a trefoil knot — the simplest topological knot of a single strand.

The significance of the new work by Stoddart and colleagues is not restricted to the synthesis of an unusual molecule. The product is constructed from components that enter into charge-transfer interactions, the basis of conducting (and superconducting) organic crystals, and so has interesting electrical properties. Most notably, electrochemical experiments show that the reduction of the quadrupally charged, positive molecule (its acceptance of electrons) varies with the environment of the accepting units relative to the donor, the reduction potential depending on whether they are on the inside or outside of the donor macrocycle. Nuclear magnetic resonance experiments show that the molecule has two 'internal melting' points — a lower one at which the acceptor part (with the bipyridinium units, blue) starts to 'pirouette' around the axis defined by the interior $O-C_6H_4-O$ unit of the donor ring, and a higher one at which both rings become free to slip around one another.

Most of all, however, the work

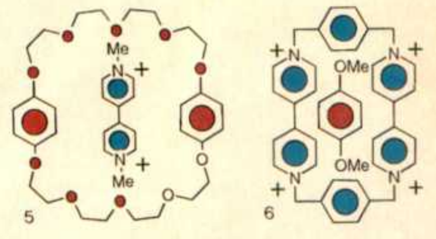


FIG. 3 Host–guest complexes of the paraquat di-cation (blue) with bisparaphenylene-34-crown-10 (red) (compound 5) and of cyclobis-(paraquat-*p*-phenylene) (blue) with 1,4-dimethoxybenzene (red) (compound 6) which were the models for the donor–acceptor forces stabilizing the intermediate in the synthesis of the [2] catenane (compound 1).

demonstrates the power of charge-transfer forces in allowing molecules to form their own template for chemical reactions. This effect lies at the very heart of the structure-directed methods of Stoddart and colleagues. If anything, Stoddart suggests there may even be an element of self-catalysis in the reaction — at least this is what seems to be indicated by the kinetics of the reaction. The authors are now working on a [3] catenane which, by all accounts, is easier to synthesize than its constituent parts. ☐

Roland Pease is an assistant editor of Nature.

1. Ashton, P.R. *et al. Ang. Chem. Int. Ed. Eng.* **28,** 1396–1399 (1989).
2. Frisch, H.L. & Wasserman, E. *J. Am. chem. Soc.* **83,** 3789 (1961).
3. Wasserman, E. *J. Am. chem. Soc.* **82,** 4433 (1960).
4. Walba, D.M., Richards, R.M. & Haltiwanger, R.C. *J. Am. chem. Soc.* **104,** 3219 (1983).
5. Ben-Efraim, D.A., Batich, C. & Wasserman, E. *J. Am. chem. Soc.* **92,** 2133 (1970).
6. Wolovsky, R. *J. Am. chem. Soc.* **92,** 2132 (1970).
7. Dietrich-Buchecker, C.O. & Sauvage, J.-P. *Chem. Rev.* **87,** 795-810 (1970).

MAMMALIAN SEX DETERMINATION

Thumbs down for zinc finger?

Paul S. Burgoyne

TOWARDS the end of 1987 *Cell* published a paper by Page *et al.*[1] which convinced many (myself included) that the mammalian sex-determining gene, which directs the developing fetal gonad to form a testis, had been identified on the human Y chromosome. The gene in question encoded a 'zinc finger' protein (such proteins are believed to interact directly with DNA) and has been dubbed *ZFY* (zinc-finger Y); intriguingly, a very closely related gene, *ZFX*, was found on the X chromosome. I suspect I was not alone among those involved in trying to unravel the process of mammalian sex determination, who, on hearing this news, considered taking early retirement. Yet the months slipped by without the expected reports of *ZFY* expression in the developing fetal testis, although the equivalent mouse sequences were shown to be expressed in adult testes[2,3]. There then appeared a paper by Sinclair *et al.*[4] which revealed that *ZFY*-related sequences in marsupials were not on the Y chromosome. While some took refuge in the heresy that the Y chromosome might not be male-determining in marsupials, I abandoned for the moment thoughts of retirement to a cottage in the country. Now, two years after the discovery of *ZFY*, two papers in this issue of *Nature*[5,6] appear to exclude *ZFY*, and the mouse equivalent *Zfy*, from having a testis-determining role. (By convention, human gene labels are all capitals, whereas mouse gene labels have only the first letter as a capital. When referring to equivalent genes without respect to species I will use capitals in inverted commas — '*ZFY*', for example.)

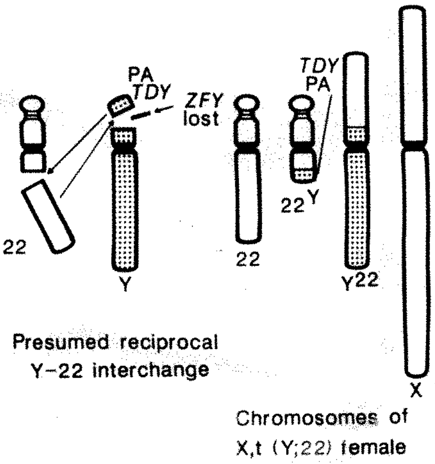
Koopman *et al.*[5] (page 940) provide the long-awaited description of '*ZFY*' expression in the developing fetal testis. In the mouse there are two Y-chromosomal homologues of '*ZFY*', which are designated *Zfy-1* and *Zfy-2*; but because it had previously been shown that testes develop in the absence of *Zfy-2* (refs 3, 7), interest centred on *Zfy-1*. The initial results obtained by Koopman *et al.* seemed to strengthen the case that *Zfy-1* is the primary testis determinant: the first *Zfy-1* transcripts appear just before testis differentiation commences, they increase in abundance as testis differentiation ensues, and then decrease once testicular differentiation is established. It is to the authors' credit that they did not rush into print at this point. Instead, bearing in mind that *Zfy-1* (and *Zfy-2*) expression in adult testes appeared to be germ-cell dependent[5], they decided to see if *Zfy-1* expression occurred in fetal testes lacking germ cells. The answer was clear — no germ cells, no *Zfy-1* expression.

Now the pattern of *Zfy-1* expression can be viewed in a very different light: the appearance and increase in *Zfy-1* transcripts is correlated with the colonization of the developing gonad with germ cells, while the subsequent decrease in *Zfy-1* expression correlates with the cessation of germ-cell proliferation and dilution of these cells with the expanding somatic components of the test[illegible]t remains to be seen how far back in the [illegible]m-cell lineage *Zfy-1* expression occurs. Because testis determination is mediated by Y-chromosome expression in the Sertoli cell lineage[8], and can occur in the virtual absence of germ cells, one must accept the authors' conclusion that their observations "exclude both *Zfy-1* and *Zfy-2* as candidates for the mouse testis-determining gene".

What of '*ZFY*' in man? Since the paper by Page *et al.* appeared it has become clear that some XX men and perhaps all XX true hermaphrodites (that is, individuals with ovarian and testicular tissue) lack *ZFY*[9], and that, in some instances, *ZFY*-negative XX males and hermaphrodites can occur in the same families. For reasons I shall come to later, I became convinced that these familial XX males and hermaphrodites could best be explained if the primary testis-determining gene was distinct from *ZFY* and lay very close to the pseudoautosomal boundary. I shall refer to this primary testis-determining gene as *TDY* (*Tdy* in the mouse). If the original 'mutation' giving rise to these familial cases of XX sex-reversal involved an X–Y interchange, then they should be positive for Y-specific sequences adjacent to the pseudoautosomal boundary.

Palmer *et al.*[6] (page 937) have examined 14 males or hermaphrodites who lacked *ZFY*; three of the males and one of the hermaphrodites did indeed carry Y-specific pseudoautosomal boundary sequences. Although the remainder lacked the boundary, they could have had small insertions that include *TDY*, rather than having mutations "elsewhere in the testis-determination pathway", as Palmer *et al.* suggest. The authors conclude that "these results lead to the strong prediction that a gene involved in sex determination lies close to or even spans the pseudoautosomal boundary". The hunt is now on!

So, do we now conclude that Page *et al.*[1] were wrong and leave it at that? I think not, because it would leave too many loose ends. Palmer *et al.* suggest that the only discrepancy between their findings and Page's lies in the latter's conclusion that at least part of the testis-determining gene must fall within the region of the Y chromosome missing in 'XY' female WHT1013. This 'XY' female in fact carried a reciprocal translocation between the Y chromosome and chromosome 22 (Fig. 1). Presumably loss of the region of the Y chromosome that includes *ZFY* occurred in conjunction with this reciprocal exchange. The crucial question is whether this individual is female because of the deletion of *ZFY*, or because of some other effect of the translocation. Palmer *et al.* favour the second explanation; they suggest that the testis-determining gene (located near the pseudoautosomal boundary) could have been inactivated through a 'position effect' because this



PA
TDY
ZFY lost
22
Y
Presumed reciprocal Y–22 interchange
TDY
PA
22
22^Y
Y^{22}
X
Chromosomes of X,t (Y;22) female

FIG. 1 The deduced arrangement of Y-chromosomal material (shaded) in the 'XY' [46,X,t(Y;22)] female WHT1013 of Page *et al.* (see ref. 1). Note that the Y pseudoautosomal region and, according to Palmer *et al.*[6], the testis-determining gene (*TDY*), are on the 22^Y chromosome. *ZFY* has been lost during the exchange.

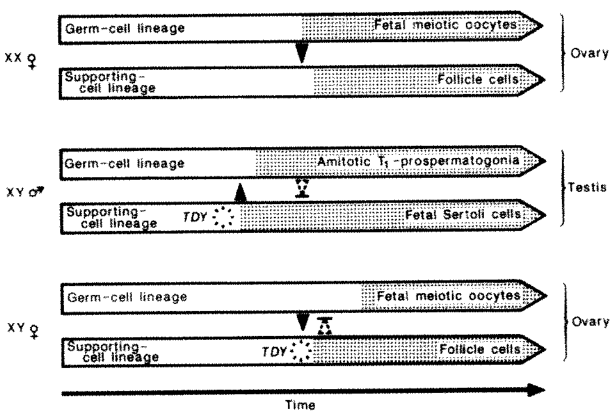


FIG. 2 A model for mammalian sex-determination and XY sex-reversal. In the 'default' pathway to ovarian development (top panel), the germ-cell lineage, as a normal consequence of its differentiation, produces a diffusible ovary determinant which commits the somatic supporting cell lineage to form follicle cells. In the diagram the production of the ovary determinant is arbitrarily shown to occur around the time the germ cells enter meiosis. In the male (second panel) *TDY* directs the somatic supporting-cell lineage to form fetal Sertoli cells. It is envisaged that these cells retain the capacity to respond to the ovary determinant. However, the fetal Sertoli cells carry out a 'pre-emptive strike' against the germ cells, committing them to the male pathway as T_1-prospermatogonia, thereby blocking the production of the ovary determinant. In the text it is suggested that one route to XY sex-reversal is via a developmental delay in the gonadal soma, so that the germ-cell lineage produces the ovary determinant *before* the pre-emptive strike from the supporting-cell lineage can occur (lower panel). The involvement of altered growth rates in sex-reversal has been argued by Mittwoch[18], although with a different mechanism to that illustrated here.

part of the Y is now attached to chromosome 22. In what follows I will try to argue a case for the alternative possibility: that deletion of *ZFY* was, as Page *et al.* supposed, the cause of the sex reversal.

In arguing the case for a role for '*ZFY*' in sex determination in man (but not in the mouse), my starting point is the striking difference between the two species in '*ZF*' gene expression in somatic tissues. In humans *ZFX* and *ZFY* are ubiquitously expressed, and *ZFX* is not dosage-compensated by X-chromosome inactivation[10]. Thus the normal expressed '*ZF*' gene dose is two – (2 *ZFX*, or 1 *ZFX* + 1 *ZFY*). In mice, only *Zfx* is expressed in somatic tissues, and it is almost certainly dosage-compensated by X-chromosome inactivation, so that XX cells have one active copy of the gene (R. Lovell-Badge, personal communication). This dosage compensation makes sense because it equalizes the '*ZF*' dosage in males and females, the normal expressed '*ZF*' dose in mice being 1 *Zfx*. Consider now that mouse XO individuals show only a mild retardation early in embryonic life[11], and have normal prenatal and postnatal viability. In marked contrast, human XO individuals are so seriously affected that more than 95 per cent of those conceived do not survive to term, and those that do are subject to the range of defects which constitute Turner's syndrome.

It does not require a major leap of imagination to attribute the severe developmental abnormalities in human XO individuals to their deficiency in '*ZF*' gene dosage, and to attribute the anti-Turner effect of the Y (men have one X chromosome but do not have Turner's syndrome) to the observed expression of *ZFY* in somatic tissues. Indeed, this anti-Turner effect of the Y maps to the same region as *ZFY*[12]. In mice, where the norm is one expressed dose of *Zfx*, XO individuals will only be at a disadvantage in the early embryonic period, when, before X-inactivation, XX embryos could be expressing two doses of *Zfx*.

I now want to introduce a concept relating to XY sex-reversal which will be crucial in the arguments that follow. The obvious route to XY sex-reversal is through loss or inactivation of the testis-determining gene '*TDY*', and there is tantalizing mention of just such a mouse mutant at the end of the paper by Koopman *et al.*[5]. But an alternative route, for which there is convincing evidence in the mouse[13,14], is one where there is a 'timing mismatch' between the testis-determination process and the ovary-determination process, such that '*TDY*' fails to pre-empt ovarian development. My own working hypothesis for how this might operate is explained in Fig. 2. This hypothesis predicts that if the development of the XY gonadal soma is slowed relative to the germinal component, then an ovary can develop. Although '*TDY*' is present it 'misses the boat'.

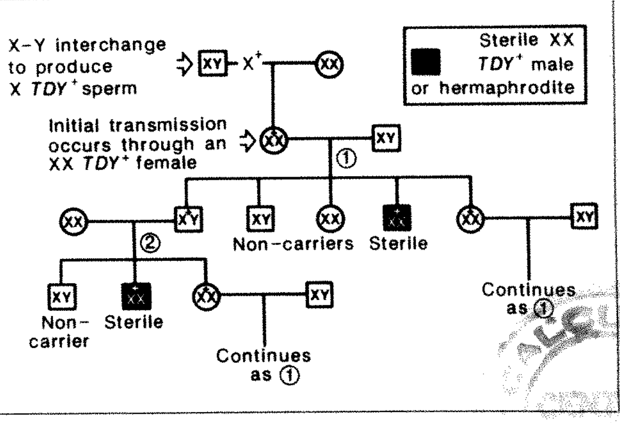
There are some human pedigrees in which the pattern of inheritance of XY sex-reversal clearly implicates an X-linked mutation. Paradoxically, in the mouse where the extensive breeding of X-chromosome-marked stocks should easily detect such a mutation, no X-linked cases of XY sex-reversal have been identified. A resolution to this paradox can be provided by bringing together the arguments presented in the preceding two paragraphs. If a double-expressed '*ZF*' dose is needed for normal human somatic development, and retardation of the somatic compartment of an XY fetal gonad can lead to sex-reversal, then a mutation that interfered with human *ZFX* expression could result in XY sex-reversal by the 'timing mismatch' route. In the mouse, where a single expressed *Zfx* dose is the norm, interference with *Zfx* expression would be expected to be lethal in early embryogenesis, thus precluding this as a route to XY sex-reversal.

It is now only a small step to explaining the sex-reversal of Page's *ZFY*-negative 'XY' female WHT1013. In man, *ZFY* contributes to the expressed '*ZF*' dose in somatic tissues, so deletion of *ZFY* could result in retardation of the gonadal soma, and thus once again lead to XY sex-reversal by the 'timing mismatch' route.

Having concluded that a double '*ZF*' dose is necessary in man to ensure that *TDY* can pre-empt the ovarian pathway, we can now return to the familial cases of *ZFY*-negative XX males and hermaphrodites. I would argue that testicular tissue develops in these individuals because they not only have *TDY* (located close to the pseudoautosomal boundary) but they also have a double 'ZF' dose (2 *ZFX*). These familial cases raise two further questions, however: how can the trait be transmitted when XX males are always sterile, and why are there so many hermaphrodites?

A lesson we have learned from mice is that hermaphroditism can only be achieved by walking a developmental tightrope: the system is strongly canalized to give either ovaries or testes. Indeed, hermaphrodites frequently have an ovary on one side and a testis on the other, rather than ovotestes. Where a particular

FIG. 3 Proposed scheme for the inheritance of *TDY*-positive, *ZFY*-negative XX sex-reversal. It is suggested (see text) that XX, *TDY*$^+$ individuals can develop not only as sterile males and hermaphrodites, but also occasionally as fertile females, the latter holding the key to the transmission of the trait.


X–Y interchange to produce X TDY$^+$ sperm
XY
X$^+$
XX
Sterile XX
■ TDY$^+$ male or hermaphrodite
Initial transmission occurs through an XX TDY$^+$ female
XX
XY
①
XX
XY
XY
XX
XX
XX
XY
②
Non-carriers
Sterile
XY
XX
XX
XY
Non-carrier
Sterile
Continues as ①
Continues as ①

genotype does produce some hermaphrodites, genotypically identical males and females are also produced. Thus I would argue that familial transmission of XX sex-reversal occurs in the first instance through a *TDY*-positive female. In the next generation transmission is possible through XY male carriers (Fig. 3). But if this hypothesis is correct, we have to explain not only how you can get *TDY*-positive hermaphrodites, but also how *TDY*-positive females arise.

I suppose that by analogy with T16/X*Sxr* mice, which can develop as females, males and hermaphrodites[15,16], a spreading of X-inactivation into *TDY* could be invoked as the cause of the variation in sexual phenotype (in these mice, the X chromosome bearing the sex-reversing factor *Sxr* is held in the inactive condition by a translocation between the X chromosome and chromosome 16). The fact that *ZFY*-positive sex-reversed XX individuals are invariably male would then have to be attributed to the interposition of more Y chromosomal material between *TDY* and the X chromosome in these cases. An alternative explanation for the occurrence of *TDY*-positive females, which I find attractive, brings us back once more to *ZFY*. Although I have argued that *ZFY* and *ZFX* are functionally interchangeable in the soma, the proteins they encode are not quite identical. It is therefore possible that *ZFY* could exert a stronger effect than *ZFX*. If so, 1 *ZFX* + 1 *ZFY* could be necessary to ensure that *TDY* pre-empts the ovarian pathway, 2 *ZFX* representing a borderline dose in this respect so that *TDY* sometimes 'misses the boat'. The *ZFY*-positive XX individuals should always be male because, in addition to *TDY*, they have overkill in terms of '*ZF*' dosage (2 *ZFX* + 1 *ZFY*).

So far we have only viewed '*ZFY*' as a '*ZFX*' substitute. What then is the evidence for '*ZFY*' having any truly male-specific role? The high levels of '*ZFY*' expression in adult testes are certainly a strong indication of a male-specific function, and I have suggested elsewhere[17] that *Zfy* (principally *Zfy-2*) could be the mouse spermatogenesis gene *Spy*; *Spy* is required from about one week after birth for the normal proliferation and survival of spermatogonia. The evidence that *Zfy-1* is expressed in the germ cells of fetal XY mouse gonads does not necessarily imply a male-specific role for the Y in the germ line at this earlier stage. XO germ cells in XO/XY mosaics, despite lacking *Zfy-1* and *Zfy-2*, certainly form fetal prospermatogonia, and go on to form definitive spermatogonia post-natally. Perhaps *Zfy-1* in the fetal XY gonad is once again just standing in for *Zfx*. After all, both X chromosomes become active in the germ line of females around this time.

The results now published by Koopman *et al.*[5] and Palmer *et al.*[6] apparently exclude '*ZFY*' from being the testis-determining gene. I have argued t[illegible]FY in man, could, together with *ZFX*, be necessary to ensure that the testis-determining gene can pre-empt ovarian development. If this is correct, then the human Y-chromosomal 'testis-determining factor' *TDF* can be equated with *TDY* + *ZFY*.

If the views presented here prove to have any validity, then many people who have shared their ideas with me deserve credit. I would especially acknowledge the contributions of Robin Lovell-Badge and Jean Weissenbach. If this all proves to be nonsense, there is always that cottage in the country. □

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1. Page, D.C. *et al. Cell* **51**, 1091–1104 (1987).
2. Mardon, G. & Page, D.C. *Cell* **56**, 765–770 (1989).
3. Nagamine, C.M. *et al. Science* **243**, 80–83 (1989).
4. Sinclair, A.H. *et al. Nature* **336**, 780–783 (1988).
5. Koopman, P., Gubbay, J., Collignon, J. & Lovell-Badge, R. *Nature* **342**, 940 – 942 (1989).
6. Palmer, M.S. *et al. Nature* **342**, 937–939 (1989).
7. Mardon, G. *et al. Science* **243**, 78–80 (1989).
8. Burgoyne, P.S. *et al. Development* **102**, 443 (1988).
9. Ramsay, M. *et al. Am. J. hum. Genet.* **43**, 4 (1988).
10. Schneider-Gadicke, A., Beer-Romero, P., Brown, L.G., Nussbaum, R. & Page, D.C. *Cell* **57**, 1247 (1989).
11. Burgoyne, P.S, Tam, P.P.L. & Evans, E.P. *J. Reprod. Fert.* **68**, 387 – 393 (1983).
12. Levilliers, J. *et al. Proc. natn. Acad. Sci. U.S.A.* **86**, 2296 – 2300 (1989).
13. Burgoyne, P.S., Buehr, M. & McLaren, A. *Development* **104**, 683 – 688 (1988).
14. Eicher, E.M. & Washburn, L.L. *A. Rev. Genet.* **20**, 327 – 360 (1986).
15. Cattanach, B.M., Evans, E.P., Burtenshaw, M.D. & Barlow, J. *Nature* **300**, 445–446 (1982).
16. McLaren, A. & Monk, M. *Nature* **300**, 446–448 (1982).
17. Sutcliffe, M.J. & Burgoyne, P.S. *Development* **107**, 373 – 380 (1989).
18. Mittwoch, U. *J. theor. Biol.* **137**, 445 – 455 (1989).

VOLCANO PREDICTION

Measures of little gravity

Robert I. Tilling

THE measurement and mapping of anomalies in the Earth's gravity field constitute a powerful geophysical technique for studying crustal structure. The detection of minute changes in the gravity field preceding volcanic eruptions is an aspect of especial interest. The causal link between changes and eruption is often hard to identify unambiguously, but Rymer and Brown, reporting on page 902 of this issue[1], suggest that a mechanism is discernible for microgravity changes preceding the eruption last spring at Poás Volcano in Costa Rica. The variations reflect, they argue, the shallow intrusion of magma beneath the volcano's summit crater lake and its subsequent cooling and vesiculation, and the evaporation of lake water. More importantly, the authors imply that the trend of gravity increase that started in 1985 was a precursor of the explosive eruption in April–May this year.

Although gravity measurements were attempted in the late 1930s at Mont Pelée (Martinique, Lesser Antilles) in an effort to identify a precursory phenomenon, it was not until the development of high-precision gravity meters in the 1950s that successful studies of temporal variations could be made[2]. With modern high-sensitivity instruments and well-designed field-measurement techniques, gravity changes at a volcano can be measured to a precision of10^{-5} (10 microgal) or better[3-6]. After correction for Earth tides and instrumental factors, measurable variations in gravity can be attributed to displacements of the observation point resulting from deformation produced by the intrusion or withdrawal of subsurface magma and the redistribution of mass in the volcanic plumbing system.

Fortunately, the free-air effect of elevation change on the observed gravity can be calculated if the elevation is also monitored. Alternatively, it can be estimated indirectly by periodic reoccupation of geodetic networks deployed to monitor ground deformation at volcanoes. Once the elevation changes are allowed for, the gravity residuals, if any, can be interpreted in terms of various models of mass redistribution affecting the volcanic system[2,3-7]: surface and subsurface movement of magma, particularly the filling and draining of a near-surface magma reservoir; changes in density of the magma bodies, through degassing, vesiculation (cavitation), cooling and contraction, or hydrothermal alteration; and changes in the surface and subsurface hydrological and geothermal regimes.

Poás is one of several active volcanoes in Costa Rica. Virtually all of its historical eruptions have occurred at a highly acid, hot crater lake that lies at its summit. Accordingly, the activity of the volcano — the boiling of lake water and its eruption in geysers, the ejection of mud plumes, explosive, steam-driven (or phreatic) eruptions, and (less commonly) the ejection of pyroclastic sulphur and lava eruptions — reflects the complex interaction, at relatively shallow depth, between the crater lake, the subsurface hydrothermal system, and the magma body[8]. In recent decades, several Costa Rican research groups, in cooperation with earth scientists from elsewhere, have initiated or augmented seismic, geodetic and other studies at Poás. Rymer and Brown's measurements of gravity changes since 1979 contribute to the growing body of knowledge about the behaviour of Poás.

The gravity changes in the period 1979–85 (less than 120 microgal) show no particular trend, permitting the somewhat speculative interpretation that the variations result largely from 'cyclical' changes in density of the underlying magma related to degree of vesiculation[1,7]. Since 1985, there has been a trend of increasing gravity (by up to 400 microgal) at some of the gravity stations, which Rymer and Brown attribute to a shallow magma intrusion which perturbed the hydrothermal activity of the crater lake. Ultimately, they suggest, this culminated in the vigorous phreatic explosions of April–May 1989. This interpretation, and the supporting data for it, are clearly summarized in figures 1-3 of their paper[1].

Unfortunately, as Rymer and Brown acknowledge, the elevation monitoring at Poás Volcano was poor before 1987. The authors argue that elevation changes before 1987 were probably not significant. But without the data, and the consequent allowances in interpreting the gravity data, their model, although certainly plausible, cannot be substantiated.

The elevation controls since 1987 need to be considered in the context of other monitoring data from Poás, one of the best-observed volcanoes in Latin America. In addition to continuous seismic monitoring, various geodetic (electronic tiltmeter, precise-level line, and ground-tilt arrays), thermal and geochemical measurements are made periodically at Poás[9]. But such data largely remain unpublished. The limited data that have been reported through the Scientific Event Alert Network (Smithsonian Institution, Washington DC) suggest that measurable changes took place in the state of the volcano leading to the April–May 1989 explosions. For example the summit was sharply inflated during June–August 1988, deflation following in the next five months; seismicity increased by an order of magnitude after January 1989; and there was a shift in the site of vigorous thermal activity in March 1989, suggesting[10] that "magma is rising below the lake . . . could lead to an eruptive event". These observations need to be incorporated with Rymer and Brown's to strengthen or modify their interpretation.

Can microgravity be used for predicting volcanic eruptions? For now, the strict answer has to be "no": a 'prediction' is generally defined[11] as "a comparatively precise statement of the time, place, and ideally, the nature and size of impending activity" made before the activity occurs. Although the microgravity studies at Poás and other well-studied volcanoes clearly show that the method is capable of detecting and monitoring magma movement, they do not yet meet the full rigour of this definition. I would fully concur with Rymer and Brown[1] that microgravity surveys have "considerable potential for understanding magma chamber dynamics and eruptive precursors, *especially when* [my italics] combined with accurate height control", if the words *only if* were substituted for *especially when*.

Lastly, microgravity surveys require the operator to be on site — not necessarily a felicitous situation for the operator given the explosive nature of the activity at many volcanoes. Techniques suited to remote and continuous monitoring seem preferable for recording precursory processes immediately before an eruption. Nonetheless, experience indicates that volcano monitoring is best achieved by integrating many approaches, among which microgravity measurements should definitely be included. ☐

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1. Rymer, H. & Brown, G. *Nature* **342**, 902–905 (1989).
2. Eggers, A.A. *J. volc. geotherm. Res.* **33**, 210 (1987).
3. Jachens, R.C. & Eaton, G.R. *J. volc. geotherm. Res.* **7**, 225–240 (1980).
4. Duzurisin, D. *et al. J volc. geotherm. Res.* **7**, 241–269 (1980).
5. Johnson, D.J. in *Volcanism in Hawaii* (eds Decker, R.W. *et al.*) *US Geol. Surv. Prof. Paper* 1350, 1297–1306.
6. Rymer, H. *Geophys. J.* **97**, 311–322 (1989).
7. Rymer, H. & Brown, G. *Bull. Volc.* **49**, 389–398 (1987).
8. Casertano, L. *et al. Bull. Volc.* **49**, 588–598 (1987).
9. *Directory of Volcano Observatories* (World Organization of Volcano Observatories, Reykjavik, 1988).
10. *Bull, Scientific Event Alert Network* **14**, 4–6 (1989).
11. Swanson, D.A. *et al. J. Geodynamics* **3**, 397 (1985).

IMMUNOLOGY

Making antigen-receptor genes

Gary Rathbun, Frederick W. Alt and George D. Yancopoulos

IN a paper that appears on page 934 of this issue[1], and in a report in *Cell*[2], two groups describe the cloning of genes that encode proteins that may play critical roles in the assembly of antigen-receptor variable-region genes. The enormous diversity of antigen-binding specificities expressed by B and T cells of the immune system depends upon a strictly regulated, site-specific genomic recombination process. This recombination process assembles component gene segments (*V* for variable, *D* for diversity and *J* for joining) into variable-region genes that encode the antigen-binding portions of the various B- and T-cell antigen receptors (Ig and TCR, respectively). *V*-(*D*)-*J* recombinase activity has been assayed in cells through the use of introduced recombination substrates (containing unrearranged *V*, *D* or *J* segments flanking selectable marker genes). Such experiments showed that a single, shared *V*-(*D*)-*J* recombinase activity rearranges all three Ig and the four known TCR antigen-receptor variable-region gene loci; the recombinase is targeted to the respective gene coding segments by similar recombination recognition sequences (RS) that flank all Ig and TCR *V*, *D* and *J* coding segments. In addition, recombinase activity was generally found only in the early stages of B- and T-cell development and not in non-lymphoid cells (for review see refs 3 and 4). Based on this information, several strategies have been employed in the attempt to isolate the genes and/or the protein products involved in *V*-(*D*)-*J* recombination. These have included isolation of genes specifically expressed in early B- and T-lineage cells, isolation of genes encoding RS sequence-binding proteins, and isolation of genes that confer a recombinogenic phenotype on non-lymphoid cells.

Recombination recognition sequences consist of a palindromic heptanucleotide sequence and an A/T-rich nonanucleotide sequence separated by a spacer of either 12 or 23 base pairs. *V*-(*D*)-*J* recombinase activity apparently only efficiently recognizes and joins a gene segment flanked by an RS with a 12-base pair spacer to a segment flanked by an RS with a 23-base pair spacer (12/23 rule). Complete understanding of the precise mechanism of *V*-(*D*)-*J* joining awaits the definition of the enzymatic machinery that carries out the process, although general aspects of this mechanism have been elucidated[3,4]. The various activities required include recognition of the RS sequences, site-specific endonucleolytic cleavage between these RS sequences and the adjacent gene segments, potential nucleotide addition and removal activities at the ends of the severed segments (which increases coding diversity at the junctions between the segments), and ligation of the gene segments involved[3]. Apart from the enzyme terminal deoxynucleotidyl transferase, thought to be involved in adding extra nucleotides during junction formation and not to be essential for *V*-(*D*)-*J* joining, none of the component(s) involved in *V*-(*D*)-*J* recombination has been isolated.

Several groups have previously reported proteins that bind specifically to the heptamer or nonamer of the RS sequences[5,6]. Matsunami *et al.*[1] now describe the purification from nuclear extracts of a precursor B-cell line of a protein of relative molecular mass 60,000 (60k) that binds to an RS, and the isolation of a complementary cDNA clone (*RBP-2*) based on the partial amino-acid sequence of this protein. The protein encoded by this cDNA does not have extensive overall homology with any known proteins, but displays an intriguing homology to a 40-residue region that is conserved among a subset of site-specific recombinase (the integrase family) in prokaryotes and the Flp recombinase in yeast. The authors suggest that this 40-residue region may represent the catalytic domain of *V*-(*D*)-*J* recombinase, with other regions of the proteins conferring binding specificity. But elucidation of the relationship of *RBP-2* to *V*-(*D*)-*J* recombinase activity awaits further information (see later).

Schatz, Oettinger and Baltimore[2] have isolated a 'recombination activating gene' (*RAG-1*) through its ability to activate *V*-(*D*)-*J* recombination when introduced into NIH3T3 fibroblasts[7]. The fibroblasts contained a recombination substrate that conferred drug resistance only when specifically rearranged by *V*-(*D*)-*J* recombinase; oligonucleotide-tagging of transfected genomic DNA fragments allowed for the isolation of the activating gene. Significantly, expression of *RAG-1* precisely correlates with expected (or measured) early lymphoid specificity of *V*-(*D*)-*J* recombinase activity in cell lines and normal tissues. The *RAG-1* gene encodes a 119k protein that is highly conserved between species. The only suggestive homology of *RAG-1* with known proteins was to the DNA-binding domain of the glucocorticoid receptor. Although these authors favour the idea that *RAG-1* encodes all or part of the *V*-(*D*)-*J* recombinase, they do not rule out the equally interesting possibility that *RAG-1* acts as a regulatory molecule that activates the *V*-(*D*)-*J* recombinase gene or protein.

Fibroblasts that express a transfected *RAG-1* gene do not express any of several other lymphoid-specific molecules, suggesting that either *RAG-1* is a very specific regulator of *V*-(*D*)-*J* recombinase or that it is a direct participant in the actual recombination process. In the second case, *RAG-1* would either act alone or recruit more ubiquitous accessory factors present at least in the NIH3T3 cell line used; there is a precedent for this, in that accessory factors are known to exist in prokaryotic systems[8]. Various analyses have indicated that the severe combined immunodeficient (SCID) mouse has a defect that impairs the final stages of *V*-(*D*)-*J* recombination[3,4,9]. The SCID mutation maps to chromosome 16, whereas *RAG-1* apparently maps to a different chromosome. Thus the SCID mutation may affect a gene normally activated by *RAG-1* (if it is an activator) or a gene encoding an accessory factor recruited by *RAG-1* (if *RAG-1* is a major *V*-(*D*)-*J* recombinase component). In support of the second possibility, recent evidence suggests that the SCID gene may encode a protein more generally involved in DNA repair[10].

Although a transfected *RAG-1* gene activates *V*-(*D*)-*J* recombination in a cell line that does not normally undergo rearrangement, it does so very inefficiently. This finding could be explained in several ways. The most mundane is that the current *RAG-1* clone carries a debilitating lesion. A more interesting explanation is that there is a lymphoid-specific component of *V*-(*D*)-*J* recombinase in addition to *RAG-1*, and that component must also be expressed for complete recombination activity to occur. If the second possibility were true, it would point to the presence of another gene encoding an activity that would complement *RAG-1* to yield higher efficiencies in the *V*-(*D*)-*J* recombination assay — the *RBP-2* gene will be an obvious candidate to test in this context.

There are many other important questions that should readily be answered with the reagents at hand. It is essential to know whether *RBP-2* actually has recombinase activity and whether *RBP-2* gene expression and/or *RBP-2* binding activity is restricted to cells with known *V*-(*D*)-*J* recombinase activity. With respect to the various possibilities outlined here, does *RAG-1* activate expression of *RBP-2* (which may then act alone or together with other factors to mediate *V*-(*D*)-*J* recombination), or does *RBP-2* act synergistically with *RAG-1* to carry out recombination? If either or both *RAG-1* and *RBP-2* are components of *V*-(*D*)-*J* recombinase, then an *in vitro* system to study *V*-(*D*)-*J* recombination may finally be feasible. Such a system should allow complete elucidation of the mechanism of the *V*-(*D*)-*J* recombination process (for example, the nature of the 12/23 joining restriction). But it remains to be seen whether the availability of *V*-(*D*)-*J* recombinase genes and gene products will provide a system better to address the nature of the mechanisms that direct a common *V*-(*D*)-*J* recombinase to join different Ig and TCR loci (with similar or identical RS sequence) in a stage- and lineage-specific manner[3]. Finally, it will be important to determine how the *RAG-1* and *RBP-2* genes evolved and whether these sequences will allow identification of related, but as yet undescribed, proteins that could act in other recombinational processes. ☐

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1. Matsunami, N. *et al. Nature* **342**, 934 – 937 (1989).
2. Schatz, D.G., Oettinger, M.A. & Baltimore, D. *Cell* **59**, 1035 – 1048 (1989).
3. Blackwell, T.K. & Alt, F.W. *J. biol. Chem.* **264**, 10327 – 10330 (1989).
4. Lewis, S. & Gellert, M. *Cell* **59**, 585 – 588 (1989).
5. Aguilera, R.T. *et al. Cell* **51**, 909 – 917 (1987).
6. Li, M., Morzycka-Wroblewska, E. & Desiderio, S.V. *Genes Dev.* **3**, 1801 – 1813 (1989).
7. Schatz, D.G. & Baltimore, D. *Cell* **53**, 107 (1988).
8. Craig, N. *A. Rev. Genet.* **22**, 77 – 105 (1988).
9. Schuler, W. *et al. Cell* **46**, 963 – 972 (1986).
10. Philips, R.A. & Fulop, G.M. *Curr. Top. Microbiol. Immun.* **152**, 11 – 18 (1989).

DAEDALUS

Lifeless prose

JUST as the calculator has produced its own brand of innumeracy, so the word processor is generating its own style of illiteracy. Its speed and ease, says Daedalus, are filling books, papers, journals and commercial literature with increasingly muddled English.

Spelling checkers, and even the more recent grammar-sensitive 'style checkers', only give the muddle a little extra polish. The reader is still left with the thankless task of trying to make sense of it all. So DREADCO programmers are developing a novel word program. It tries to deduce what, if anything, the author has to say, and then nags him into saying it clearly.

DREADCO's 'Literary Critic' seeks clarification of every sentence typed. "You haven't mentioned protons before", it might remark; or "You said the opposite in line 157", "Is 'aromatic' the smell or the chemical?", and for real puzzles, "Do you really mean that?".

These seemingly intelligent interjections stem from the program's attempt to find a 'logical tree' behind the growing narrative. Like other conversational programs, the Literary Critic has a big dictionary of words and synonyms classified by type — animals, locations and so on — and connected by their most likely relationships. Once it has decided what the text is 'about', it queries every apparently new or discordant topic, and amends its dictionary accordingly. It marks occurrences with their stated sequence in time, keeps tabs on the 'cast' of concepts or characters being discussed, and asks further questions when ambiguities or inconsistencies seem to occur. Where logically necessary, it generates new linking text, and tries to arrange the whole assembly of statements into their neatest logical tree. Such is the power of persistent Socratic questioning, says Daedalus, that the most complex and rambling chain of remarks should soon be clarified into a coherent story. It won't be immortal prose, but it should be a grammatical and effective piece of writing.

Self-conscious stylists will greatly resent the Literary Critic with its endless silly questions. But the children and students of the electronic age will simply accept it as an effortless and automatic way of working out what they want to say, and then saying it in a straightforward manner. And many humbler folk, with fascinating ideas and personal stories to tell if they only dared to sit down and write them out, will be grateful for its tireless and patient tuition. It will help policemen and insurance assessors to extract sensible evidence from befuddled witnesses, tease effective instructions out of skilled but inarticulate craftsmen, and trick unwary criminals into much completer confessions than they had intended.

David Jones

molecular-genetic information is usually introduced at this point, followed by the clinical aspects, including at least the principles of treatment. The general sections have been considerably expanded to take account of the needs of students at all levels, as well as of scientists and clinicians working in the field, to be conversant with many areas of molecular biology, cell biology, immunology, major chromosomal disorders and cytogenetics.

The field covered by the book has expanded greatly as more single-enzyme defects have been defined and as the genetic heterogeniety of those already known has been documented. Heterogeniety within the inborn errors of metabolism also stems from the fact that the specific mutation operates against different backgrounds of polygenic influences and environmental factors in different individuals. There are also genetic factors contributing to diseases that are not inherited in a simple mendelian manner but that operate through biochemical mechanisms. These considerations indicate further expansion in the future and emphasize the masterly degree of compression which has been achieved in this new edition.

Appropriately, most writers on inherited single-enzyme defects and human biochemical variation, including the present editors, look back to Sir Archibald Garrod's Croonian lecture "Inborn Errors of Metabolism", delivered before the Royal College of Physicians of London in June 1908, as a landmark in the development of their subject. The present growing confluence of the biomedical sciences, providing a substratum for our wider understanding of disease, but defying the old categorizations such as biochemistry, molecular biology, genetics and pathology, was perhaps foreshadowed in Garrod's essay "The Inborn Factors in Disease", published in 1931. In this undeservedly little-known work, Garrod explored the concept of diathesis (a condition of the body which renders it liable to certain diseases) and tried to discern some elements of the scientific basis for it.

As the last decade of the twentieth century dawns, it seems reasonable to suggest that macromolecular structure interactions and aberrations (macromolecular pathology) are the scientific bases for diatheses, and a type of explanation towards which Garrod was groping more than 60 years ago. The sixth edition of *The Metabolic Basis of Inherited Disease* points forward in this direction and hence towards a wider brief for future editions. ☐

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The practice of theory

M. B. Usher

Mathematical Biology. By J. D. Murray. *Springer-Verlag: 1989. Pp. 767. DM89, £35, $59.*

In a minority of books, the author strikes the 'right' note and the reader says something like "I couldn't put it down". Most books do not fall into this category even when the author has something important to say. Other books, usually works of reference, simply defy reading because of the sheer magnitude of the task. Murray's book falls into this category. That is not to belittle the book in any way, as it is clearly a *magnum opus* on the application of mathematics in biology. Nevertheless, I have dipped into it rather than reading it from cover to cover, omitting some sections and rereading others. In the areas of biology that interest me, I have discovered here some fascinating analyses.

The book is divided into 20 chapters, starting with ecology (growth of single-species populations) and ending with epidemiology (here meaning the geographical spread of diseases, although the definition could also include the spread of invasive species). The beginning and end do, however, obscure the pathway that Murray takes from one to the other. Perhaps it could be said that the continuous growth of single-species populations produces the simplest model ($dN/dt \propto N$) and hence is the best starting point. Murray then expands this simple model into discrete population models for single species and into both continuous and discrete models for interacting populations. To me, the surprising feature of this section of the book is that it is set in a calculus mould; Murray does not include the equivalent formulation of discrete models as Leslie matrices and the subsequent developments of difference equations.

Murray then moves from populations to reaction kinetics, which are introduced with a detailed analysis of Michaelis–Menten theory. Biological oscillators, feedback control mechanisms and the Hodgkin–Huxley theory of nerve membranes serve as an introduction to a section on the many facets of oscillators. As might be expected, this section includes a discussion of limit cycles and of local/global stability, but it also includes topics such as unexpected 'black holes' in this area of biology.

Oscillator-generated wave phenomena are used to introduce the study of biological waves; together with the concept of reaction–diffusion the book then explodes into a bewildering array of applications, cutting right across the traditional divisions in biology. Models of waves are used to explore the spread and control of insect populations, the calcium-activated waves across the eggs of amphibia and a teleost fish, and patterning in cultures of slime mould. I found the analyses in chapter 15 particularly fascinating because the reaction–diffusion mechanisms are used to explore mammalian coat patterns (hence the spotted leopard on the cover of the book) and, more excitingly I felt, the patterns on butterfly wings. Although some, perhaps most, patterns of lepidopteran wings can be modelled on the basis of a diffusing morphogen, it is salutory that a series of butterfly wing patterns is illustrated for which models have yet to be developed. Is this a project for a PhD student?

Pattern is not manifest solely in the morphological patterns of mammals, insects or marine algae, but also in relation to neurobiology and development. Although in chapter 16 Murray largely concentrates on drug-induced hallucinations, he also explains mathematically how sufferers of migraine headaches see geometrical patterns as a result of instabilities in neural activity in the visual cortex. He also applies these models to the patterns found on mollusc shells.

Murray concludes with two chapters on epidemics. The favourite old examples — black death, venereal diseases and rabies — are all there, with the inevitable addition of AIDS and other newer diseases. Murray applies the model for the spread of rabies to the so-far hypothetical example of what would happen if the disease were to reach Britain. Assuming that an epidemic starts near the port of Southampton, he predicts that the wave front will reach London in approximately two years and the south of Yorkshire, in the north of the country, in approximately four years.

Murray has produced a magnificent compilation of mathematical models and their application in biology. I am sure that I shall refer to the text frequently, though I feel uncertain about attempting the exercises that conclude each chapter. The text is a fine exposition of calculus models in the biological sciences. I do, however, have some misgivings: the first three words that I looked up in the index were 'matrix', 'fractal' and 'chaos'. 'Matrix' is not there, but to be fair neither is 'differential equation'. 'Fractals' are not there either, but 'chaos' is discussed now and again, mainly in relation to the discrete logistic model. I regret that the book is not as comprehensive as I at first hoped, but nevertheless, couched in terms of calculus, there is a wealth of fascinating examples here. ☐

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Making a real discovery

T. Cavalier-Smith

Prochloron: A Microbial Enigma. Edited by Ralph A. Lewin and Lanna Cheng. *Chapman and Hall: 1989. Pp. 129. £42, $53.*

EIGHTY years ago, Mereschkowsky suggested that chloroplasts of green plants and red, brown and other algae evolved from differently coloured symbionts that independently took up residence inside the cells of different protozoa. Although the origin of chloroplasts from prokaryotic (bacterial) symbionts has been firmly established for about a decade, it is still very uncertain whether chloroplasts evolved several times, as Mereschkowsky argued, or only once or twice.

Mereschkowsky's theory of multiple origins was given a considerable boost in 1975, when Lewin and Cheng showed that the photosynthetic green cells symbiotic in certain colonial seasquirts living on mangrove roots, sea shells and corals were not eukaryotic green algae, as previously thought, but gigantic green bacteria. What was most exciting about these bacteria was not their exceptional size (9–30 micrometres, a size more typical of eukaryotic cells) but the fact that they differed from cyanobacteria and resembled instead green-plant chloroplasts in two important respects: replacement of the blue or red phycobilin pigments by chlorophyll *b*; and the stacking of their photosynthetic (thylakoid) membranes. After first thinking of them as blue-green algae (now more often called cyanobacteria) Lewin therefore renamed these unusual green cells *Prochloron*, and placed them in a separate division which he called Prochlorophyta.

Many people concluded that Lewin and Cheng had discovered another of Mereschkowsky's hypothetical symbionts and that green-plant chloroplasts had evolved from *Prochloron* or some other prochlorophyte, whereas the phycobilin-containing chloroplasts of red algae and Glaucophyceae had obviously evolved from cyanobacteria. Expeditions to Pacific islands to collect material to test this idea have resulted in the publication of nearly 100 papers, whose conclusions are lucidly summarized in this excellent and valuable book.

Virtually everything significant known about *Prochloron* and its symbiosis with seasquirts is included, together with beautiful colour plates of the fascinating variety of didemnid seasquirts that harbour *Prochloron*. In several cases the seasquirts clearly gain nutritionally from cultivating *Prochloron* in their bodies. The main evolutionary conclusion to be drawn is that *Prochloron* is really not as fundamentally different from cyanobacteria as was once thought, and is probably not specifically related to the chloroplasts of green plants. Both Lewin and Stackebrandt now tend to accept the view, long argued by Chadefaud and myself, that *Prochloron* evolved from a cyanobacterium (by the loss of phycobilisomes and the associated evolution of chlorophyll *b* and thylakoid stacking) quite independently of green plant chloroplasts. Alberte suggests some possible selective advantages for these changes.

The recently discovered free-living prochlorophyte *Prochlorothrix*, to which the final chapter is devoted, also appears not to be specifically related to green chloroplasts. Because of the possibility of convergent multiple losses of phycobilisomes and origins of chlorophyll *b* (which differs from *a* in only one atom), one cannot even be sure that the prochlorophytes are monophyletic. It also remains possible, though perhaps unlikely, that green plants obtained their chloroplasts from the same cyanobacterium as did red algae but that euglenoids got theirs from a prochlorophyte. The discovery of a third unnamed type of prochlorophyte suggests that prochlorophytes could be more diverse than initially thought: even *Prochloron* should probably be split into at least three species. Atypical photosynthetic bacteria continue to be discovered sufficiently frequently to suggest that still more surprises may lie ahead.

Both Lewin and Stackebrandt touch on the problem of classifying the prochlorophytes. This group obviously belongs in the Eubacteria, not in the plant kingdom, despite Lewin's original creation of the botanical division Prochlorophyta. But the likelihood that these organisms evolved from cyanobacteria is insufficient reason to include them within the same family as cyanobacteria, as Stackebrandt suggests; that would be confusing and would undervalue their distinctiveness. The paraphyletic and exceedingly diverse class Photobacteria clearly should be abolished, as Stackebrandt implies. But instead one should treat Cyanobacteria and Prochlorobacteria (a better name than Prochlorophyta) as classes within a single new division — the Phycobacteria ('algal' bacteria) — which would include all those bacteria sharing the property of oxygenic photosynthesis with their descendants the chloroplasts. Phycobacteria differ sufficiently profoundly from purple and green anoxygenic bacteria to be a separate division.

An unusual and valuable feature of the book is the inclusion of abstracts of all the papers that have been published on *Prochloron*, which nicely complement the more discursive chapters on its cytology, biochemistry, symbiosis and phylogeny. The editors' very practical, and sometimes amusing, hints for collecting and handling *Prochloron* complete a first-rate work, the first devoted to the Prochlorobacteria. ☐

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Sitting on the fence

David Lindley

Cold Fusion: The Making of a Scientific Controversy. By F. David Peat. *Contemporary Books; 1989. Pp.188. $16.95.*

YOU would think that anyone writing about the cold fusion affair would be sensitive to the dangers of hasty publication. Not F. David Peat, apparently. Like Stanley Pons and Martin Fleischmann, who went public on the basis of what turned out to be highly disputable results, Peat has produced an account of cold fusion based on skimpy research and incomplete data. He also shies away from pronouncing judgement. This may be partly because his story comes to a halt during September 1989, when it was still possible (with a little effort) to imagine that there might be something to Pons and Fleischmann's claims, but is more a consequence of Peat's complete reluctance even to try to sift from the assertions and counter-assertions any measure of truth.

Peat's telling of the story adds little to what anyone who followed the comprehensive newspaper and magazine coverage will already know. Even so, Peat makes some mistakes with his chronology: he conflates the Materials Research Society meeting in San Diego, at which Robert Huggins unconvincingly defended his claim for excess heat, with the Electrochemical Society meeting in Los Angeles, at which Pons and Fleischmann similarly defended their claim for excess heat.

Nor is Peat's account of the science of cold fusion very penetrating. Two chapters of the book are occupied with routine expositions of nuclear physics, 'hot' fusion, solar energy, the chemistry of palladium and so on. Observing the quantity of theoretical ideas thrown out in response to Pons and Fleischmann's claim, Peat says "theoretical physicists and chemists appear to have no problem in accounting for cold fusion", a sunny judgement presumably based on the notion that a thousand completely wacky ideas weigh about the same as one or two moderately plausible ones. But in order to maintain some critical distance, the author adds "only time will tell how well [the theories] stand up to a closer examination".

The problem with Peat's book is not that it is conspicuously wrong anywhere, but that it is so stubbornly uninformative. Throughout the tale there are disputes, disagreements and contradictions, all of which offer the author opportunities to dig deeper, unearth the truth and in the process bring to light some aspect of the nature of scientific discovery or the character of the participants. But at every such opportunity, Peat refrains from comment and moves hastily on. "For some scientists," he writes near the beginning, "the Fleischmann and Pons announcement produced a sense of elation. But for others, it was a matter of skepticism." Seven chapters later, having followed the controversy through a number of rowdy scientific meetings and heard most of the relevant pros and cons, we reach Peat's final, balanced evaluation: "Cold fusion may lead nowhere. Or we may be standing on the threshold of something extraordinary." ☐

David Lindley is on the editorial staff of Nature.

Clock watching

Owen Gingerich

Empires of Time: Calendars, Clocks, and Cultures. By Anthony Aveni. *Basic Books: 1989. Pp. 371. $24.95.*

"Time, like an ever-rolling stream, bears all its sons away", runs a line in the familiar hymn. But is time really, objectively, like an ever-flowing stream, or is that just a Western cultural notion? Such is the central question of this aptly titled book from the astronomer turned anthropologist Anthony Aveni.

At the heart of Aveni's discourse are three chapters dealing with Mayan, Aztec and Incan time. The author is obviously in command of this material, which has been close to his research interests for more than a decade. These time systems, devoid of borrowings from Western civilization, enable him to raise the "big question of human nature": given basically the same stimuli, do different people in different places all react to what they see in the same way? The answer is loud and clear: the intricately interlocking calendars of the Maya or the geometrically land-mapped calendars of the Incas are far removed from our own views of time, so wildly different that even writers of science fiction could hardly have dreamed them up.

Aveni focuses his account on these pre-Columbian civilizations by first considering a fascinating group of tribal societies — the Mursi and Nuer in Africa, the Bororo from central Brazil and the Trobrianders from New Guinea, these last with their edible timekeeping palolo worm. (These marine annelids spawn every year following the full moon in mid-autumn, allowing the Trobrianders to keep their lunar calendar in synchronism with the solar seasons.) Aveni's discussion ranges from basic biological rhythms, to the history and vicissitudes of the Western calendar, and even to a romp through modern cosmology. Although the book is somewhat uneven in quality, these topics offer an intriguing and enlightening menu for the general reader.

As a practicing archaeoastronomer, Aveni takes a decidedly sceptical view of the claims of nascent science at Stonehenge but, he asks, "Isn't there a bit of science in a ritual conducted at a time of year signaled by the passage of sunlight down an avenue or through a stone archway?". One might wish that he had evinced even more scepticism: Aveni cites reports that potato metabolism anticipates barometric pressure by two days, mentions that the Latin word *mens* signified "moon", and reckons that the Age of Aquarius will begin in AD 2137. (By my own calculations, it began well over a century ago.) It is also hard to believe his claim that the gregorian calendar reform was antisemitic.

These quibbles aside, Aveni has put together a well-written and handsomely illustrated volume. His exploration of the mysteries of time provides an eminently stimulating read, wonderfully suitable for the Christmas break. ☐

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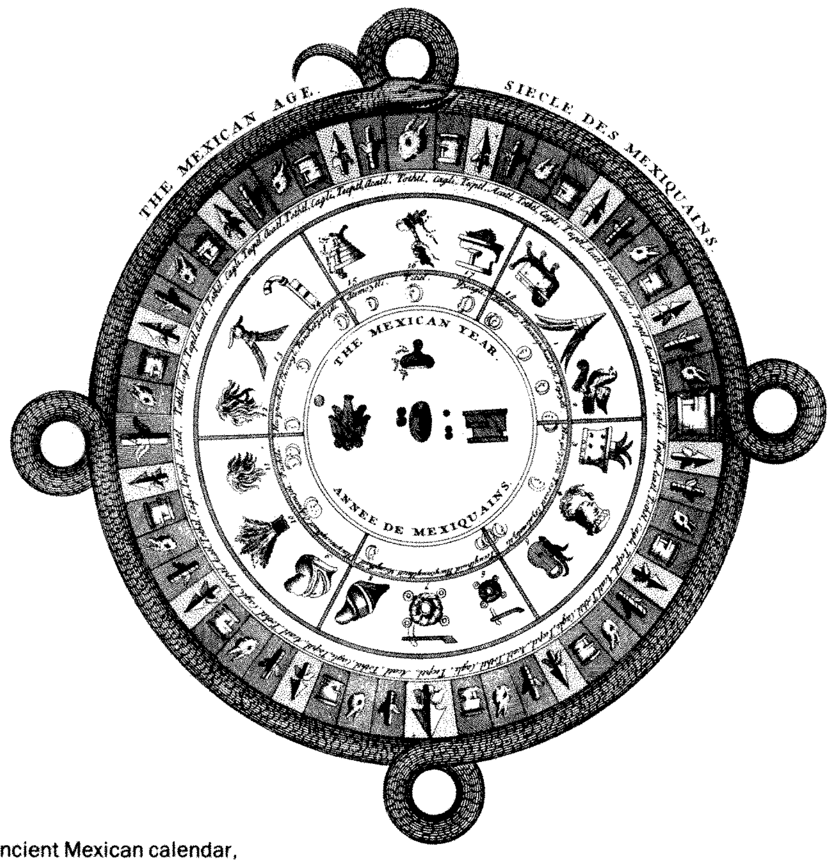
THE MEXICAN AGE.
SIECLE DES MEXIQUAINS
THE MEXICAN YEAR.
ANNEE DE MEXIQUAINS

Ancient Mexican calendar,
are differences in interpretation linked to culture and civilization?

Mary Evans Photo Library

New in paperback

- First published in 1988, *Time in History* by G. J. Whitrow is available in paperback. The volume discusses the general influence of time on the mental outlook and way of life in different ages and civilizations, as well as the history of the measurement of time. Published by Oxford University Press, price £5.95, $8.95.
- *The Dread Disease: Cancer and Modern American Culture* by J. T. Patterson is now issued in paperback by Harvard University Press, price £11.95, $14.95 (see review in *Nature* **330**, 285; 1987).
- *Electricity and Magnetism* Volumes I and II, by B. I. Bleaney and B. Bleaney, are available as a 3rd edn in paperback from Oxford University Press. Price Vol. I £9.95, $19.95; Vol. II £13.50, $26.95.
- *Universality in Chaos*, 2nd edn, by P. Cvitanović has been updated by the addition of several articles reflecting the developments in universality theory. Published by Adam Hilger, price £15.

Distilled delight

D.H. Perkins

The Experimental Foundations of Particle Physics. By Robert N. Cahn and Gerson Goldhaber. *Cambridge University Press: 1989. Pp. 428. £30, $49.50.*

PROGRESS in physical science depends on the close interplay between experiment and theory, and nowhere has this been more true than in the physics of elementary particles. Many standard texts on this subject tend to emphasize the beauty, conciseness and all-embracing nature of theoretical principles and ideas, so it is a pleasure to review a text in which, for a change, discussion of experiments takes pride of place.

Cahn and Goldhaber describe key experiments in twelve areas of particle physics, covering the discovery, over the years, of the various lepton and quark constituents of matter and the nature of the interactions between them. The authors have struck an excellent balance in what must have been a difficult choice of material.

The book is intended for advanced undergraduates and graduates in physics. The text in each chapter introduces a particular area of the field, presenting the basic physical concepts and background information necessary for an understanding of the significance of, typically, three or four original key papers, which are reprinted at the end of each chapter. The contents range from discovery of the neutron, positron, muon and pion in the 1930s and 1940s, to the study of "strange" particles and hadron resonances in the 1950s and 1960s, which led to the quark model and was crowned with the discovery of the massive "charm" and "beauty" states in the 1970s. There are excellent chapters on the study of weak interactions, of the strong forces between quarks, and on the discovery of neutral currents and the W and Z bosons validating the electroweak model unifying the electromagnetic and weak interactions.

I believe the authors' assessment of the role of the various experiments to be well considered, authoritative and scrupulously fair. I have to say, however, that I sometimes found the balance between the space devoted to different topics to be slightly odd. The discovery at CERN of W and Z bosons — which were a crucial prediction of the electroweak model and for which the scientific world had waited 15 years — is covered in 1½ pages of text, whereas the Stanford discoveries of charm quarks and the tau-lepton — also very important — get 20 pages. Presumably this simply reflects the north Californian origin of the authors. There are few *faux pas*, although omitting the name of Bruno Touschek in a discussion of colliders must be one of them.

Included at the end of each chapter is a set of problems. Many of these are well beyond undergraduate level and they are mostly of a theoretical nature. What a pity that, in a text devoted to the experimental foundations of the subject, there are not more practical exercises which involve calculating a real number! My only other criticism is that a little discussion of accelerator and detector technology and development would have been useful, as these form the backbone of the experimental scene. Regarding the quality of presentation, I have to say that an otherwise well-produced book was for me marred by the poor quality of reproduction of the original papers, particularly in the first three chapters.

In summary, I believe this text to represent a major achievement in collecting, analysing and distilling for the reader, material forming an intensely exciting chapter in modern science. □

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Spinning out

P.J. Hore

One and Two Dimensional NMR Spectroscopy. By Atta-ur-Rahman. *Elsevier: 1989. Pp.578. Dfl.355, $186.75.*

NUCLEAR magnetic resonance (NMR) spectroscopy has changed almost beyond recognition in the past decade. A glance through the literature reveals a truly staggering array of ingenious and specialized techniques for determining the structures and motions of molecules. Assisted by developments in computer and magnet technology, this metamorphosis has been made possible by the ease with which long-lived coherent superpositions of nuclear spin states (coherences) can be created and manipulated using pulses of coherent, monochromatic, radiofrequency radiation.

No longer are chemists content simply to monitor the equilibrium state of the nuclear spins in a molecule subject to the restrictions of spectroscopic selection rules; rather they tailor the spectrum to suit their needs, shifting spin polarizations and coherences around the molecule so as to map out its chemical structure. A crucial factor in this revolution has been the move away from the conventional one-dimensional approach. Two-dimensional NMR, in which signals are functions of two frequency variables, is now routine, while the first forays into a third frequency dimension have occurred in the past two years. The relief of crowding in the spectra of complex molecules afforded by two-dimensional methods, together with their ability to reveal correlations between nuclei, have led to previously undreamt of achievements, most notably the determination of the complete three-dimensional structure of small proteins.

To take the greatest advantage of these new NMR techniques, the chemist needs to understand the physics underlying them. Atta-ur-Rahman's book is one of a handful of recent publications that aim to provide such insight, while also offering advice on the choice of experiment and guidance on interpretation of spectra. The book is restricted to proton and carbon-13 NMR of liquids, and deals with essentially every significant experiment up to about 1986. An undergraduate knowledge of NMR is assumed, but not much else. Most chapters finish with exercises in spectral interpretation chosen from the author's own research in natural product chemistry.

Atta-ur-Rahman succeeds in conveying the scope and power of one- and two-dimensional NMR and shows clearly how structural information can be extracted. But he is less convincing when discussing how radiofrequency pulse sequences actually work. This he does by means of Felix Bloch's semi-classical vector model which, although simple and pictorial, cannot satisfactorily handle multiple quantum coherence or coherence transfer. As most modern techniques involve one or both of these elements, this is really a severe limitation. A newcomer to modern NMR would surely be better advised to embrace the product operator formalism to which the author devotes his final, short chapter. Once mastered, this approach requires only simple algebra and determination to enable the inner workings of complex experiments to be seen.

Sadly, there is a sufficient number of typographical errors to be irritating and, in places, confusing or misleading. Nor is the reader helped by a poorly organized introductory chapter and a rather imprecise style of writing. In short, this is a comprehensive and much-needed book, but it has serious flaws. □

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■ Two new volumes in the series *Methods in Enzymology* bear witness to the remarkable transformation in the practical application of NMR spectroscopy in the past 10 years. Volume 176, *Nuclear Magnetic Resonance* part A, covers basic techniques and methods for studying protein dynamics. Volume 177 (part B) describes protein modifications. Both volumes are published by Academic; part A is $65, £46.50, part B (to be published in January 1990) is $65, £46.50.

The role of mantle plumes in the development of continental drainage patterns

K. G. Cox

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The geomorphology of three continental flood-basalt provinces supports a recent model for the generation of such provinces by melting of hot mantle plumes. Characteristic drainage patterns indicating topographic doming associated with plume activity are still preserved after up to 200 Myr. Crustal thickening by magmatic underplating is the most likely cause of the persistence of such features.

THE plume hypothesis was first applied to continental areas as an explanation of flood-basalt volcanism by Burke and Dewey[1]. In its new version White and McKenzie[2] postulate the former existence of plumes with specific sizes and locations, and it is this aspect of the model that I shall test. The model postulates that plumes of hot mantle material rise as relatively narrow columns that spread laterally at a high level to produce areas of anomalously hot asthenosphere up to 2,000 km across. When a plume rises beneath a continent the dynamic and thermal effects may produce up to 2 km of surface uplift in the centre of an extensive dome. Fracturing of the dome by gravitational forces may produce rifts and massive amounts of basaltic volcanism as the anomalously hot mantle undergoes decompressive melting. If conditions are favourable the rift may widen and a new ocean will be formed. Plume activity itself produces uplift that is strictly temporary, but even after it has ceased, the continental areas affected characteristically remain topographically high, a feature that has been ascribed to crustal thickening caused by magmatic underplating[3,4].

The plume hypothesis provides a framework in which many features of the geology of non-orogenic continental areas can be considered, and here I present geomorphological evidence that provides strong support for the model.

Drainage patterns over continental swells

The idea of great continental uplifts (swells) with rifted crests is an old one (see, for example, ref. 5). Figure 1 shows Cloos' drawing[5] of the Afro-Arabian dome, split by the Red Sea, Gulf of Aden and Ethiopian rifts. This figure illustrates the fundamental components of the morphology: outwardly tilted blocks separated by rifts.

If continental break-up takes place, a typical continental flood-basalt province in a particular continent ought initially to display a morphology appropriate to (roughly) half of such a structure. The unrifted flanks of the dome should be characterized by river systems draining away from the new continental edge (dome-flank systems), whereas failed arms of the rift system should lead drainage towards the new ocean (rift-related systems). Figures 2–6 illustrate the drainage patterns of three areas of Gondwanaland affected by flood-basalt volcanism during and at the end of the Mesozoic. The circles are taken directly from White and McKenzie's diagrams and indicate their estimates of the extents of the plume tops. I believe these figures speak for themselves in showing a remarkable general correspondence between postulated plume locations and the occurrence of the predicted drainage patterns. The details (especially the extent to which the patterns deviate from the strictly radial and the extent to which they may have been influenced by other factors such as more linear uplifts along rift shoulders or along the continental margins) will be discussed elsewhere.

The Deccan

The Deccan province[6] (Fig. 2) is the youngest of the three, having formed at about the time of the Cretaceous/Tertiary boundary[7,8]. The west coast of peninsular India was formed at about the same time as the eruption of the traps by the separation of India and the Seychelles bank[9]. This has been interpreted as due to a ridge jump as India passed over a plume, now believed to be situated beneath Reunion[10]. Deccan geomorphology and uplift have been described[11-13]. The present western margin of peninsular India is marked by the escarpment of the Western Ghats, the northern half of which is cut into the Deccan basalts. Most rivers rise close to the west coast (sometimes within as little as 50 km) and drain eastwards into the Bay of Bengal, although in the Malwa region in the north, drainage is north-easterly into the Ganga valley. I interpret the peninsula as a whole as the flank of the remaining eastern half of the original dome. I assume that if an opposing half existed, that it has been disrupted by crustal thinning and now lies below sea level in the Seychelles and the Saya de Malha bank.

In the north, examples of exceptional drainage, towards the new ocean, are provided by the west- or south-flowing fault-controlled valleys of the Tapti, Narmada and Gulf of Cambay systems[14,15], which are interpreted as failed rift arms.

Southern Brazil

The coast of southern Brazil was formed in the Lower Cretaceous[16,17] by the opening of the South Atlantic. Co-eval volcanism is represented now by the extensive basalts and rhyolites of the Paraná Basin[18] (the Serra Geral Formation) and

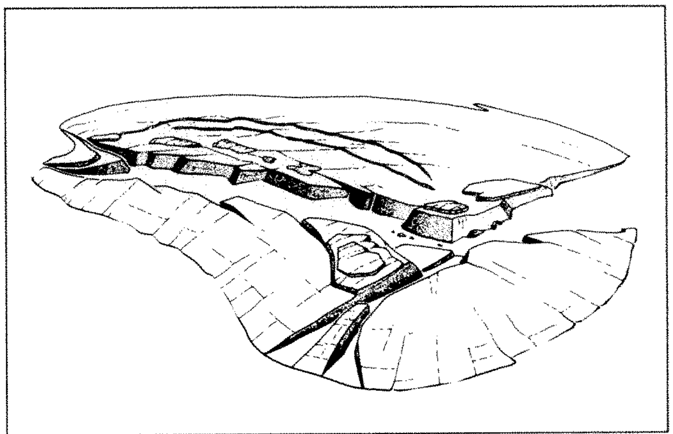

FIG. 1 The Afro-Arabian dome, redrawn from ref. 5. The view is from the south with Africa in the foreground, separated from Arabia by the Red Sea (left) and the Gulf of Aden. The Ethiopian rift system cuts the African side of the dome in the foreground. If the drawing is turned upside down a remarkable similarity is observed between the African side and the morphology of peninsular India (Fig. 2), with the Ethiopian rift playing the part of the Narmada and Tapti rifts.

by a relatively small opposing outlier on the African side at Etendeka in Namibia[19] (Figs 3 and 4). In Brazil the coastal escarpment (the Serra do Mar) rises steeply for a distance of ~1,500 km between Porto Alegre in the south and Vitoria in the north, and the plateau edge inland attains heights of ~2,000 m above sea level. Geomorphologically this area is similar to the Deccan, the predominant drainage flowing westwards, directly away from the new ocean. Unlike the Indian case, however, the presence of the Andes results in the inward drainage being collected by the Paraná river system and returned to the Atlantic by the River Plate, at a point well to the south of the Paraná basalt outcrop. On the African side, only small areas of the corresponding volcanics are preserved, but there is an extensive coast-parallel topographic high, stretching from the northern part of the Cape Province of South Africa through Namibia and Angola. This directs much of the drainage away from the coast into the Kalahari Basin. I interpret the Serra do Mar and Cape-Angola topographic highs as representing the opposing halves of a single original structure.

Unlike the Deccan the Paraná dome does not seem to have been endowed with failed-rift arms.

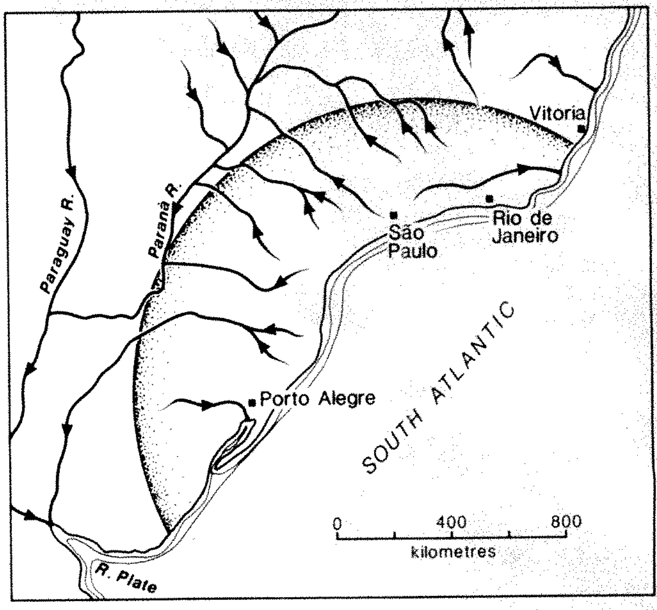

FIG. 3 Drainage pattern of southern Brazil with superimposed plume[2]. Except in the south near Porto Alegre, dome-flank drainage is dominant.

The Karoo

In southeastern Africa the main peak of volcanism, associated with the separation of Africa and Antarctica, took place in the Lower Jurassic[20] and the topographic signature is more difficult to read. The area covered by remnants of the volcanism, the Karoo volcanic province, is huge, extending in the north-east from southern Malawi and the vicinity of Mozambique Island, to the north-west at Mariental in Namibia, and thence southwards over the remainder of the continent. Rocks of equivalent age and composition are found in the Dronning Maud Land area of Antarctica, where clearly the other 'half' of the province is located. Ice-cover, however, precludes consideration of Antarctic topography in the present work.

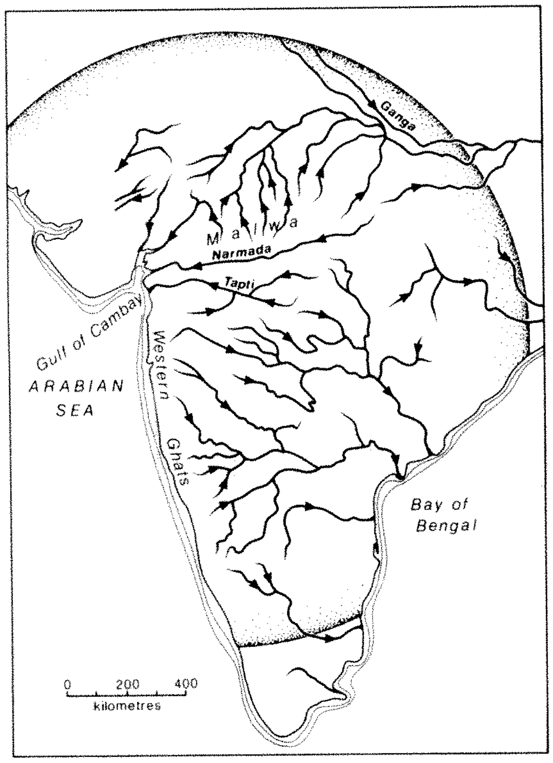

FIG. 2 Drainage pattern of peninsular India with the postulated Deccan plume[2] superimposed (stippled circle). Most of the peninsula preserves dome-flank drainage, also seen in the Malwa area in the north. The Gulf of Cambay, Narmada and Tapti systems consist of rift-related drainage. The westernmost left-bank tributary of the Tapti illustrates river capture of the dome-flank drainage by the rift-related system.

Because of its great extent in Africa and because of the spatial overlap of the Karoo province with the southern end of the East African rift system, it is not immediately clear which topographic features should be related to the proposed Karoo plume. High ground broadly parallels the east coast, running from Natal in the south (the Drakensberg) through the eastern Transvaal and the eastern highlands of Zimbabwe into southern Malawi and neighbouring parts of Mozambique. Maximum elevation is reached in the heavily dissected mountains of Lesotho (Thaba Ntlenyana, 3,500 m) but there are large tracts of plateau country at ~2,000 m above sea level in the Orange Free State and the Transvaal. All the high ground mentioned lies within White and McKenzie's postulated plume top.

Two major rivers, the Limpopo and the Zambezi, flow eastwards into the Indian ocean, interrupting the coast-parallel topographic high. Both are zones of Karoo-age rifting[1,21], and hence analogous to the Narmada in the Deccan province. The greater age of the Karoo province, however, has resulted in considerable extension of the drainage basins of these rivers, leaving the dome-flank sectors much reduced. There are still two areas, however, where the characteristic drainage patterns are well preserved.

A pattern analogous to that of southern Brazil and peninsular India is still obvious in the southern part of the province (Fig. 5). Here there are no rifts to disturb the simple pattern of westward drainage off the crest of the Drakensberg into the Orange River and Vaal River systems. The Orange River rises only 150 km from the south-east coast but flows into the Atlantic at Oranjemond, 1,400 km due west (Fig. 4), so it is evidently a classic case of drainage directed away from the new ocean. The dome-flank drainage is also excellently preserved in north-western Zimbabwe where the right-bank tributaries of the Mid-Zambezi flow with sub-parallel courses to the north-west, at right angles to the Zambezi itself (Fig. 6).

The evidence from Africa supports White and McKenzie's positioning of the Karoo plume and its areal extent. It is not obvious, however, that the Antarctic side of the plume is realistic in their presentation because it takes no account of volcanism through the Transantarctic mountains (see, for example, refs 22, 23 for further discussion).

Antiquity of drainage patterns

Major continental drainage patterns have often been postulated as being of considerable antiquity[24] although they are obviously difficult to date (see summary in ref. 25). Here the extremely close spatial correspondence between proposed plume locations and predicted drainage systems indicates a direct genetic relationship and, by implication, that the river systems originated at the same time as the plume activity. Thus for example, the Orange system must have originated in the Jurassic, probably at or at about the time of the Lower Jurassic peak of Karoo activity[26] about 190 Myr BP (before present). Between the Kalahari and Oranjemond, the Orange cuts a considerable gorge through the Cape–Angola high. The river is <200 m above sea level, but is closely bounded, especially to the north, by substantial tracts of country at elevations of 1,500 m or more. The Orange is clearly antecedent to the Cape–Angola high, a conclusion consistent with the postulated Jurassic age of the drainage system, and the implied Lower Cretaceous age of the high, as the eastern half of the Paraná dome.

The Orange thus predates the South Atlantic and presumably originally flowed on into the general region of Buenos Aires, lending weight to speculation[25] about the possibility of other African rivers such as the proto-Congo flowing across South America.

Surface uplift

The plume model involves initial uplift of the existing land surface, in response to the dynamic effects of the plume. This may have initiated the drainage patterns observed, yet they still exist, and in all cases the postulated plumes are now far from the provinces concerned. White and McKenzie argue that further magmatic material accretes to the base of the crust during plume activity (the phenomenon of underplating) and that the resulting crustal thickening causes the basalt provinces to remain topographically high in the long term. This hypothesis is based on petrological arguments[27] and is supported by wide-angle seismic experiments in the vicinity of rifted areas (some of which have been drilled), but it is difficult to test directly. For further studies of this problem, however, it is important to try to quantify the uplift.

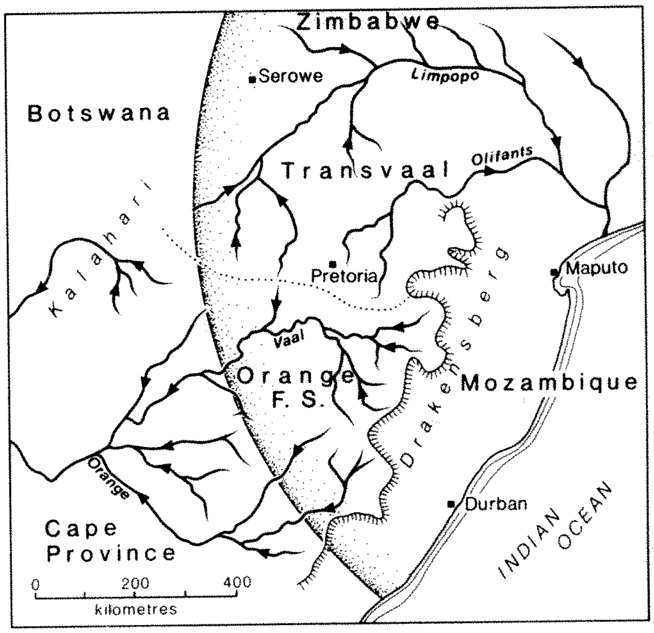

FIG. 5 Drainage patterns in southeastern Africa, with Karoo plume superimposed. Dome-flank drainage is preserved west of the Drakensberg escarpment (Orange River and Vaal systems). The dotted line separates dome-flank drainage in the south from rift-related (Limpopo) drainage in the north. Note the extreme enlargement of the catchment of the latter compared with the Narmada–Tapti system in Fig. 2.

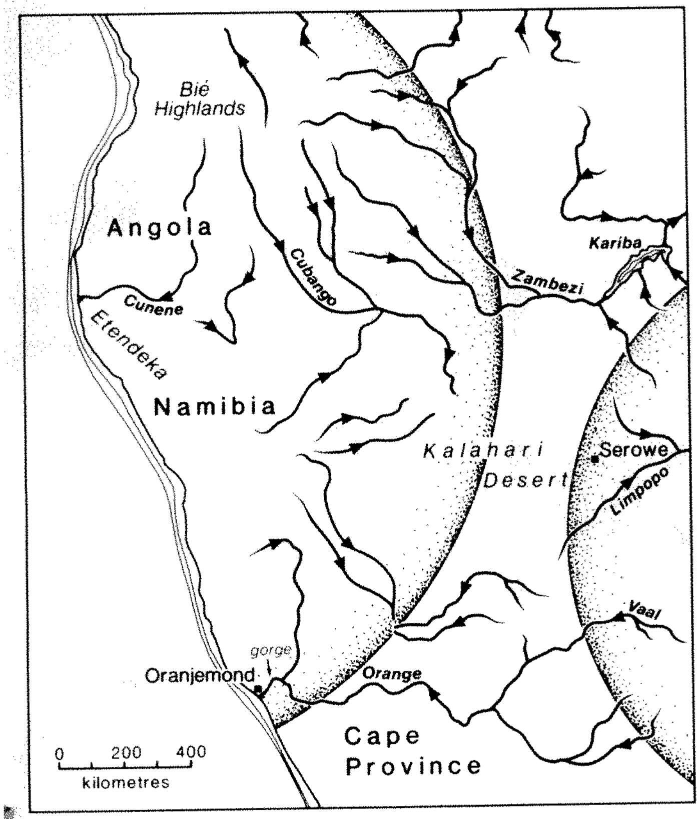

FIG. 4 Drainage patterns of the Cape–Angola topographic high, with the Paraná plume (left) and part of the Karoo plume (right). An irregular dome-flank pattern is preserved draining eastwards into the Kalahari. Note the Orange River gorge, where antecedent drainage cuts the younger uplift.

Surface uplift (the term is used to mean that the average elevation of the ground increases[28]) on a regional scale is difficult to demonstrate. Here I shall make only a very general argument, mainly for the Karoo province, that suggests the likelihood of about 1.5 km of post-Lower-Jurassic surface uplift for the Drakensberg region. The Palaeozoic history of a large part of Gondwanaland, particularly the western and southern regions (the Paraná Basin[29], the Karoo[30], and the Transantarctic Mountains[31]) was one of platform sedimentation. In the Lower Palaeozoic much of this showed marine influences, but by the Permian, deltaic and fluviatile environments were widespread. By the beginning of the Mesozoic a vast area had been transformed into the subaerial desert over which the Cave Sandstone was deposited (southern Africa) and, where not terminated by volcanism, these conditions persisted right through the Jurassic into the Lower Cretaceous (for example, in the Botucatu sandstones of the Paraná Basin). The Palaeozoic/early Mesozoic history is thus one of an extensive depositional basin slowly filling up and eventually becoming a subaerial desert.

After the volcanism, the whole area has behaved completely differently, and the dispersed fragments of this region of Gondwanaland have been high-standing erosional environments. This constitutes a strong general argument in favour of long-lasting surface uplift.

I assume that the Cave Sandstone desert lay fairly low at the time of initial Karoo volcanism, perhaps <1,000 m above sea level. The sub-basalt unconformity now lies at 2,000 m in Lesotho, but more importantly, over a large area to the north of this, through the Orange Free State and the eastern and northern Transvaal, although the basalts have largely been removed by erosion, the Karoo sedimentary sequence is still widely preserved. This is a plateau area unincised by major canyons, so the average elevation of large tracts is clear. Much of it approaches 2,000 m, and the implication of the Karoo geology is that the basalt unconformity lay not far above the present erosion surface. Returning to the initial assumption about the elevation of the desert, this indicates surface uplift of 1-2 km.

I have speculated that the Kalahari basin might owe its existence to the fact that it was unaffected by both the Karoo and Paraná volcanic episodes, hence remaining free of the associated uplift[20] (the 'passive basin' model[1]). Much of the basin lies 500-1,000 m above sea level. There has been both post-Jurassic erosion and deposition[30] but I speculate that the present elevation is still about the same as that of the general pre-Jurassic land surface of southern Africa. The Kalahari is just the present-day continuation of the great Cave Sandstone and Botucatu desert, and its present elevation (about 1.5 km below the Transvaal/Orange Free State plateau) gives some support to the calculation above.

Erosion surfaces

Thus far I have tried to view the geomorphological evolution of the areas discussed in terms of consequences of the initial plume activity, which is in some cases very old. Yet the geomorphological literature of the Gondwanaland continents is rich in references to cyclical-erosion surfaces and scarp retreat[32-34] and in many cases relatively recent, such as late Cenozoic, uplift events have been recognized. Partridge and Maud[34] particularly stress the episodic nature of uplift. Can the two views be reconciled? Can the whole evolution be seen in terms of a single original cause, or is it necessary to postulate a series of apparently unrelated uplift events?

A reconciliation may be found by postulating mechanical coupling between the continental crust and the newly-formed oceanic crust. After an early surface uplift has created a coast-parallel topographic high, erosion of the swell promotes rock uplift (used in the sense of England and Molnar[28] to indicate upward movement of bedrock) in response to denudation and isostatic adjustment. The continental margin is subjected to opposing stresses. Inland the bedrock is travelling upwards; on the other side the oceanic crust is, if anything, likely to founder. I assume that flexure of the lithosphere can accommodate, up to a point, these conflicting demands. Beyond that point the continental margin may respond by fracture and undergo local surface uplift. Despite the fact that rock uplift is continuous in the continental interior, scarp retreat in response to episodic marginal uplift, with concommitant changes of the base level of erosion, can generate the impression of episodic uplift throughout.

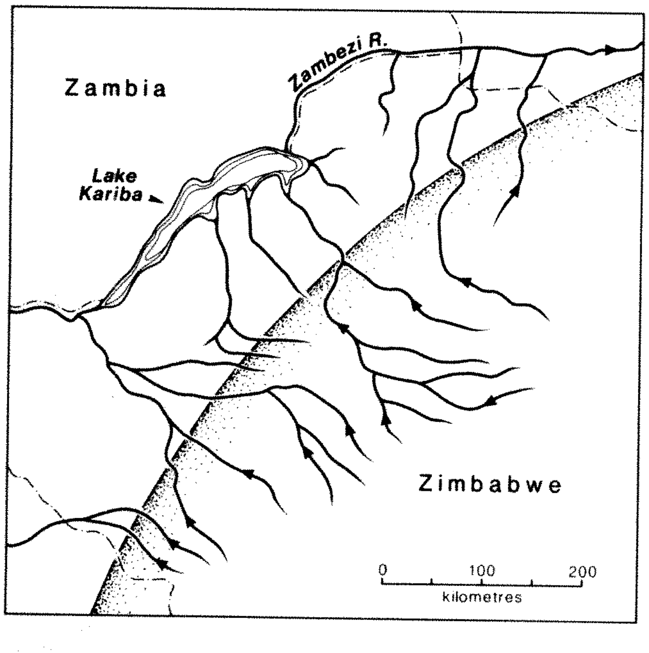

FIG. 6 Drainage in the northwestern part of the Karoo province, with part of the Karoo plume. Right-bank tributaries of the Zambezi are shown, and represent well preserved dome-flank drainage. The southeastern part of the area is occupied by left-bank tributaries of the Limpopo, the enlarged rift-related system of which the southern side is shown in Fig. 5.

Drainage-pattern preservation and plume location

After initial formation, dome-flank drainage patterns come under attack from rift-related systems. Within continental marginal zones the latter flow at low topographic levels relative to the adjacent dome-flank systems, and consequently expand their catchments by river capture. Some of the left-bank tributaries of the Tapti (Fig. 2) show this in an early stage (65 Myr after initiation), and the huge bite the Limpopo system has taken out of the Karoo dome flanks in 180 Myr is notable. Preservation is thus partly related to the age of the province, but dome-flank drainage patterns are best preserved in areas where the volcanics, or flat-lying sub-volcanic sediments, are still present or where they have only relatively recently been removed by erosion. These conditions are met by the Deccan, much of the Paraná, and the Orange Free State and Transvaal, where preservation is aided by the presence of the widespread flat-lying sedimentary rocks of the Karoo Basin. In northwestern Zimbabwe a thin veneer of basalts is still present, accounting for the very regular pattern of the mid-Zambezi right-bank tributaries.

By contrast, the Cape-Angola topographic high is deeply eroded, as the towering Jurassic/Cretaceous[35] plutons of Damaraland testify. Fission-track studies[36] indicate several kilometres of erosion during the Cretaceous. As a result the drainage system into the Kalahari basin, although still recognizable, has now adopted a more complex pattern as it adjusts to heterogeneous basement rocks. Apart from the small Etendeka remnant, whatever basalt cover was present has been completely removed.

White and McKenzie positioned their plumes using two main criteria: the centre had to coincide with the pre-drift position of a known hotspot if present, and the circle had to include the known seaward-dipping reflectors as far as possible. Here I have shown the expected drainage patterns, which support the model despite its evident simplicity. Future studies of denudation, for example by fission-track methods, and more detailed analysis of drainage patterns that are less obviously preserved, however, may enable more extensive mapping of the areas influenced by plumes. ☐

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1. Burke, K. C. A. & Dewey, J. F. *J. Geol.* **81,** 406-433 (1973).
2. White, R. S. & McKenzie, D. P. *J. geophys. Res.* **94,** 7685-7730 (1989).
3. McKenzie, D. P. *Nature* **307,** 616-618 (1984).
4. Brooks, C. K. *Z. Geomorph., Suppl.* **54,** 101-117 (1985).
5. Cloos, H. *Conversation with the Earth* (Routledge & Kegan Paul, London, 1954).
6. Mahoney, J. J. in *Continental Flood Basalts* (ed. Macdougall, J. D.) (Kluwer, Dordrecht, 1988).
7. Duncan, R. A. & Pyle, G. *Nature* **333,** 841-843 (1988).
8. Courtillot, V. *et al. Nature* **333,** 843-846 (1988).
9. Norton, I. O. & Sclater, J. G. *J. geophys. Res.* **84,** 6803-6830 (1979).
10. Morgan, W. J. in *The Sea 7* (ed. Emiliani, C.) 443-487 (Wiley, New York, 1981).
11. Radakrishna, B. P. in *Geomorphological Studies in India* (University of Sagar, India, 1965).
12. Kailasam, L. N. *Tectonophysics* **29,** 505-521 (1975).
13. Kailasam, L. N. *Tectonophysics* **61,** 243-269 (1979).
14. Murty, T. V. V. G. R. K. & Mishra, S. K. *J. geol. Soc. Ind.* **22,** 112-120 (1981).
15. Raju, A. T. R., Chaube, A. N. & Chowdhary, L. R. *Bull. Volcanol.* **35,** 521-538 (1972).
16. Siedner, G. & Mitchell, J. G. *Earth planet. Sci. Lett.* **30,** 292-302 (1976).
17. Asmus, H. E. & Baisch, P. R. *Episodes* **4,** 3-9 (1983).
18. Piccirillo, E. in *Continental Flood Basalts* (ed. Macdougall, J. D.) (Kluwer, Dordrecht, 1988).
19. Erlank, A. J. *et al. Spec. Publ. geol. Soc. S. Afr.* **13,** 195-245 (1984).
20. Cox, K. G. in *Continental Flood Basalts* (ed. Macdougall, J. D.) (Kluwer, Dordrecht, 1988).
21. Cox, K. G. in *African Magmatism and Tectonics* (eds Clifford, T. N. & Gass, I. G.) (Oliver & Boyd, Edinburgh, 1970).
22. Duncan, A. R., Erlank, A. J. & Marsh, J. S. *Spec. Publs geol. Soc. S. Afr.* **13,** 355-388 (1984).
23. Cox, K. G. *Nature* **340,** 341-342 (1989).
24. Potter, P. E. *J. Geol.* **86,** 13-33 (1978).

25. Ollier, C. D. *Tectonics and Landforms* (Longman, New York, 1981).
26. Fitch, F. J. & Miller, J. A. *Spec. Pubis geol. Soc. S. Afr.* **13,** 247–266 (1984).
27. Cox, K. G. *J. Petrol.* **21,** 629–650 (1980).
28. England, P. C. & Molnar, P. *Geology* (in the press).
29. Petri, S. & Fulfaró, V. J. *Geologia do Brasil* (Editora da Universidade de São Paulo, 1983).
30. Tankard, A. J. *et al. Crustal Evolution of Southern Africa* (Springer, New York, 1982).
31. Craddock, C. (ed.) *Antarctic Geoscience* (University of Wisconsin Press, Madison, 1982).
32. King, L. C. *South African Scenery* 2nd edn (Oliver and Boyd, Edinburgh, 1951).
33. King, L. C. *The Morphology of the Earth* (Oliver and Boyd, Edinburgh, 1962).
34. Partridge, T. C. & Maud, R. R. *S. Afr. geol. J.* **90,** 179–208 (1987).
35. Cahen, L., Snelling, N. J., Delhal, J. & Vail, J. R. *The Geochronology and Evolution of Africa* (Clarendon, Oxford, 1984).
36. Brown, R. W. *6th Int. Conf. Fission Track Dating* Abstr. Vol. Université de Franche-Comté, Besançon, 1988).

Conformations of immunoglobulin hypervariable regions

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On the basis of comparative studies of known antibody structures and sequences it has been argued that there is a small repertoire of main-chain conformations for at least five of the six hypervariable regions of antibodies, and that the particular conformation adopted is determined by a few key conserved residues. These hypotheses are now supported by reasonably successful predictions of the structures of most hypervariable regions of various antibodies, as revealed by comparison with their subsequently determined structures.

THE relationships between the amino-acid sequences of immunoglobulins and the structures of their antigen-binding sites are important for understanding the molecular mechanisms of the generation and maturation of the immune response and for designing engineered antibodies. Antigen-binding sites are formed by six loops of polypeptide, the hypervariable regions; three from the variable domain of the light chain (VL) and three from the variable domain of the heavy chain (VH), denoted L1, L2, L3, and H1, H2, H3, respectively (Fig. 1*a*). Within the domains, the loops are connected to a β-sheet framework whose structure is conserved[1,2]. The specificity and affinity of the binding sites are governed by the structures of the six hypervariable regions[3,4].

Two models can be proposed for the relationship between the amino-acid sequence and structure of the binding-site loops. In one model, different sequences produce different conformations for both the main chain and side chains of the loops. Because hypervariable regions have different sequences in different antibodies, this model implies that each region adopts a different conformation in different antibodies. In the other model, antibodies have only a few main-chain conformations or 'canonical structures' for each hypervariable region. Most sequence variations would only modify the surface provided by the side chains on a canonical main-chain structure. Sequence changes at a few specific sets of positions would switch the main chain to a different canonical conformation.

Canonical structure model

Experimental evidence indicates that the canonical structure model describes the relationship between amino-acid sequence and structure for at least five of the six hypervariable regions[5-9]. Kabat *et al.*[5] found conserved residues at sites within certain sets of hypervariable regions and suggested that they had a structural role. Padlan and Davies[6], and more recently de la Paz *et al.*[7], showed that some of the hypervariable regions in the immunoglobulins of known structure have the same main-chain conformation in spite of several differences in sequence.

Chothia and Lesk[8] identified the residues that through packing, hydrogen bonding, or the ability to assume unusual values of the torsion angles ϕ, ψ or ω, are primarily responsible for the main-chain conformations of the hypervariable regions in the structures then known—the Fab fragments of NEW (ref. 10), McPC603 (ref. 11), KOL (ref. 12) and J539 (ref. 13) and the VL domains of REI (ref. 14) and RHE (ref. 15). The conformations are determined by the interactions of a few residues at specific sites in the hypervariable regions and, for certain loops, in the framework regions. Hypervariable regions that have the same conformations in different immunoglobulins have the same or very similar residues at these sites (Fig. 1 and Table 1). Examination of the amino-acid sequence of the antibody D1.3 showed that its hypervariable regions are the same size as those in known structures and contain the same or similar residues at the sites responsible for known conformations[9]. On the basis of these observations the atomic structure of the VL–VH dimer of D1.3 was predicted before its experimental determination. Comparison of this predicted structure with the preliminary crystal structure showed that the conformations of four of the hypervariable regions had been predicted correctly; the conformation of L3 was significantly different from that predicted, and

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TABLE 1 Sequences and conformations of Vκ and VH hypervariable regions of known structure

L1 Regions†

Canonical Structure	Protein	26	27	28	29	30	31	a	b	c	d	e	f	32	2	25	33	71
					*										*	*	*	*
1	J539	S	S	S	V	S	—	—	—	—	—	—	—	S	I	A	L	Y
	HyHEL-5	S	S	S	V	N	—	—	—	—	—	—	—	Y	I	A	M	Y
	NQ10	S	S	S	V	R	—	—	—	—	—	—	—	Y	I	A	M	Y
2	REI	S	Q	D	I	I	K	—	—	—	—	—	—	V	I	A	L	Y
	D1.3	S	G	N	I	H	N	—	—	—	—	—	—	Y	I	A	L	Y
	HyHEL-10	S	Q	S	I	G	N	—	—	—	—	—	—	N	I	A	L	F
	NC41	S	Q	D	V	S	T	—	—	—	—	—	—	A	I	A	L	Y
3	McPC603	S	E	S	L	L	N	S	G	N	E	K	N	F	I	S	L	F
4	4-4-20	S	Q	S	L	V	H	S	—	N	G	N	T	Y	V	S	L	F

Total no. of sequences known for L1 regions: human, 95; mouse, 299.

Canonical structure	1	2	3	4
Human sequences that fit (%)	—	60	5	5
Mouse sequences that fit (%)	15	25	20	10

L2 Regions

Canonical Structure	Protein	50	51	52	48	64
					*	*
1	REI	E	A	S	I	G
	McPC603	G	A	S	I	G
	J539	E	I	S	I	G
	D1.3	Y	T	T	I	G
	HyHEL-5	D	T	S	I	G
	HyHEL-10	Y	A	S	I	G
	NC41	W	A	S	I	G
	NQ10	D	T	S	I	G
	4-4-20	K	V	S	I	G

Total no. of sequences known for L2 regions: human, 69; mouse, 183.

Canonical structure	1
Human sequences that fit (%)	95
Mouse sequences that fit (%)	95

L3 Regions

Canonical Structure	Protein	91	92	93	94	95	96	90
						*		*
1	REI	Y	Q	S	L	P	Y	Q
	McPC603	D	H	S	Y	P	L	N
	D1.3	F	W	S	T	P	R	H
	HyHEL-10	S	N	S	W	P	Y	Q
	NC41	H	Y	S	P	P	W	Q
	4-4-20	S	T	H	V	P	W	Q
	NQ10	W	S	S	N	P	L	Q
2	J539	W	T	Y	$\overset{*}{\mathrm{P}}$	L	I	$\overset{*}{\mathrm{Q}}$
3	HyHEL-5	W	G	R	N	$\overset{*}{\mathrm{P}}$	—	$\overset{*}{\mathrm{Q}}$

Total no. of sequences known for L3 regions: human, 52; mouse, 152.

Canonical structure	1	2	3
Human sequences that fit (%)	90	—	2
Mouse sequences that fit (%)	80	10	1

H1 had a very different fold from that predicted[9]. (We report below that the refined conformation of D1.3 corresponds more closely to the predicted structure.)

An examination of the library of the known immunoglobulin sequences shows that many immunoglobulins have hypervariable regions that are the same size as those in the known structures and contain the same or closely related residues at the sites responsible for the known conformations[8]. These observations indicate that for at least five of the hypervariable regions there is only a small repertoire of canonical main-chain conformations and that the conformation actually present can often be predicted from the sequence by the presence of specific residues.

The accuracy of the canonical structure model for immunoglobulin binding sites depends on (1) the correct determination of the sets of residues responsible for the observed conformations and (2) changes in the identity of residues at other sites not significantly affecting the conformations of the canonical structures. The model can be tested, refined and extended by using it to predict the atomic structures of binding sites in immunoglobulins before their structures have been determined by X-ray crystallography.

We have now tested the canonical structure model by using it to predict the structures of four immunoglobulins before their structures had been experimentally determined. These immunoglobulins are HyHEL-5 (ref. 16), HyHEL-10 (ref. 17), NC41 (ref. 18) and NQ10 (S.S., P.M.A. and R.J.P., manuscript in preparation). The analysis of the amino-acid sequences of these immunoglobulins indicated that 19 of their 24 hypervariable regions should have conformations close to known canoni-

H1 Regions‡

Canonical Structure	Protein	26	27	28	29	30	31	32	34	94
		*	*		*				*	*
1	McPC603	G	F	T	F	S	D	F	M	R
	KOL	G	F	I	F	S	S	Y	M	R
	J539	G	F	D	F	S	K	Y	M	R
	D1.3	G	F	S	L	T	G	Y	V	R
	HyHEL-5	G	Y	T	F	S	D	Y	I	R
	NC41	G	Y	T	F	T	N	Y	M	R
	NQ10	G	F	T	F	S	S	F	M	R
	4-4-20	G	F	T	F	S	D	Y	M	G
1′	NEW	G	S	T	F	S	N	D	Y	R
	HyHEL-10	G	D	S	I	T	D	D	W	N

Total no. of sequences known for H1 regions: human, 50; mouse, 321.

Canonical structure	1
Human sequences that fit (%)	50
Mouse sequences that fit (%)	80

H2 Regions§

Canonical Structure	Protein	52a	b	c	53	54	55	71
							*	
1	NEW	—	—	—	Y	H	G	
	D1.3				G	D	G	
	HyHEL-10	—	—	—	Y	S	G	
		*					(*)	*
2	HyHEL-5	P	—	—	G	S	G	A
	NC41	T	—	—	N	T	G	L
						(*)		*
3	KOL	D	—	—	D	G	S	R
	J539	P	—	—	D	S	G	R
	NQ10	S	—	—	G	S	S	R
						(*)	*	*
4	McPC603	N	K	G	N	K	Y	R
	4-4-20	N	K	P	Y	N	Y	R

Total no. of sequences known for H2 regions: human, 54; mouse, 248.

Canonical structure	1	2	3	4
Human sequences that fit (%)	15	1	40	15
Mouse sequences that fit (%)	15	40	5	20

The residues listed here (single-letter code) are those that form the hypervariable regions and those in the framework regions that are important for the observed conformations of these regions[8]. The hypervariable regions are taken as those outside the framework β-sheet[8]. Except for H2, they are similar to, but not identical with the regions that show high sequence variations and which Kabat *et al.*[26] use to define hypervariable regions. The sequences are grouped so that those that have the same main-chain conformation, or canonical structure, are adjacent. The canonical structure numbers used below refer to the conformations shown in Fig. 1. The residues in the hypervariable and framework regions that are mainly responsible for these conformations[8] are indicated by an asterisk. The classification and sequence requirements of the H2 conformations have been revised in the light of work described here and elsewhere[28]. For each hypervariable region the number of human and mouse sequences listed by Kabat *et al.*[26] are given. We also give the percentage of these sequences that are the same size as the known canonical structures and have the same residues at the positions marked by an asterisk.

† Canonical structure 4 is illustrated in Fig. 4. Although the size of the known L1 structures varies between 6 and 13 residues, they have closely related folds with residues 26-19 and 32 packed against the framework in the same conformation[8]. The remaining residues form a turn or loop on the surface (Figs 1 and 4). The ends of the long loops have some flexibility. There are another 25% of the human sequences and 20% of the mouse sequences that have one more residue than structure 2, or one fewer than structure 4, and whose sequences satisfy the requirements listed above. It is expected that these differ only in the conformations of the tips of the surface loops.

‡ The H1 hypervariable regions with canonical structure 1 have very similar conformations: the r.m.s. differences in the coordinates of their main-chain atoms are 0.3–0.8 Å. The H1 regions in NEW and HyHEL-10 only partly satisfy the sequence requirements for structure 1 and have a distorted version of its conformation.

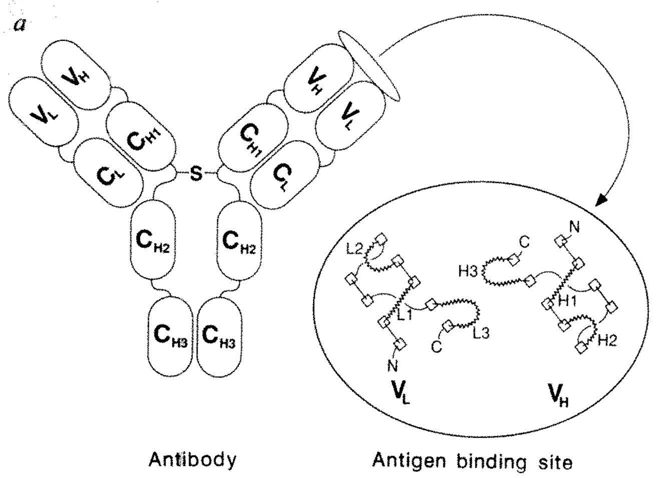
§ The H2 region here comprises residues 52a–55. The region with high sequence variation is 50–65 (ref. 26). In the known structures the main-chain conformation of 50–52 and 56–63 do not differ significantly[8] (Fig. 1*b*). (*), The residues at positions 55 or 54 in the canonical structures 2, 3 and 4 have residues with positive values for ϕ and ψ, and usually, but not in all cases, Gly, Asn or Asp is found at these sites. For a sequence to match that of canonical structure 2, 3 or 4 the presence of these residues at sites 54 or 55 is required.

cal structures. We then compared the predicted structures of these hypervariable regions with the subsequently determined structures. Another immunoglobulin structure, 4-4-20 (ref. 19) has recently been reported. We did not have the opportunity to predict the structure of 4-4-20 before its experimental determination, and we discuss here only how its hypervariable regions have the conformations expected from the known canonical structures. Also, we report that the refined conformation of D1.3 (ref. 20) corresponds more closely to the predicted structure.

Model building procedure

The main-chain conformations of the hypervariable regions in the Vκ and VH domains of known structure are shown in Fig. 1. The residues responsible for these conformations are listed in Table 1. Each hypervariable region in the immunoglobulins of unknown structure was examined to determine (1) whether it has the same size as any homologous hypervariable region of known structure and (2) whether its sequence contains the set of residues responsible for a known conformation. Except for L3 in HyHEL-5, all the light-chain regions correspond to a known canonical structure, as do all the H1 regions and the H2 region in HyHEL-10 (Table 1). The conformation predicted for the H2 regions in NC41 and HyHEL-5 was based on the analysis of the H2 region in the preliminary structure of J539 (ref. 8). In all three of these antibodies the H2 region is a four-residue turn with Gly at the fourth position and the predicted conformation is that almost always found for such turns[21]. (Below we present a more accurate analysis of H2 regions.) For H3 regions in HyHEL-5, HyHEL-10, NC41 and NQ10, no prediction of conformation could be made on the basis of the known canonical structures.

The sequences of the VL and VH domains were compared to see which of the known framework structures have sequences close to those of the unknown structures. From the comparisons of the hypervariable and framework regions, one known VL and one known VH structure were taken as the starting points—parents—for the model of the predicted structure. If the conformation predicted for a hypervariable region was not present in the parent, but was present in another known structure, the hypervariable region in the parent was replaced by that in the other structure. Side chains in the parent that were different from those in the unknown structure were replaced[22,23] and the resulting model subjected to a very limited energy refinement[24].



HyHEL-5, HyHEL-10 and NC41 hypervariable regions

The atomic structures of Fab fragments of immunoglobulins HyHEL-5, HyHEL-10 and NC41 in complexes with their protein antigens were determined by X-ray crystallography[16-18]. The resolution of the X-ray data used to determine the structures and value of the residual (R) after refinement are (complex, resolution, R value): HyHEL-5-lysozyme, 2.5 Å, 20%; HyHEL-10-lysozyme, 3.0 Å, 24%; and NC41-neuraminidase, 2.9 Å, 19%. These structures have been determined at medium resolution. The tracing of the polypeptide chain of the hypervariable regions is unambiguous, although the orientation of some of

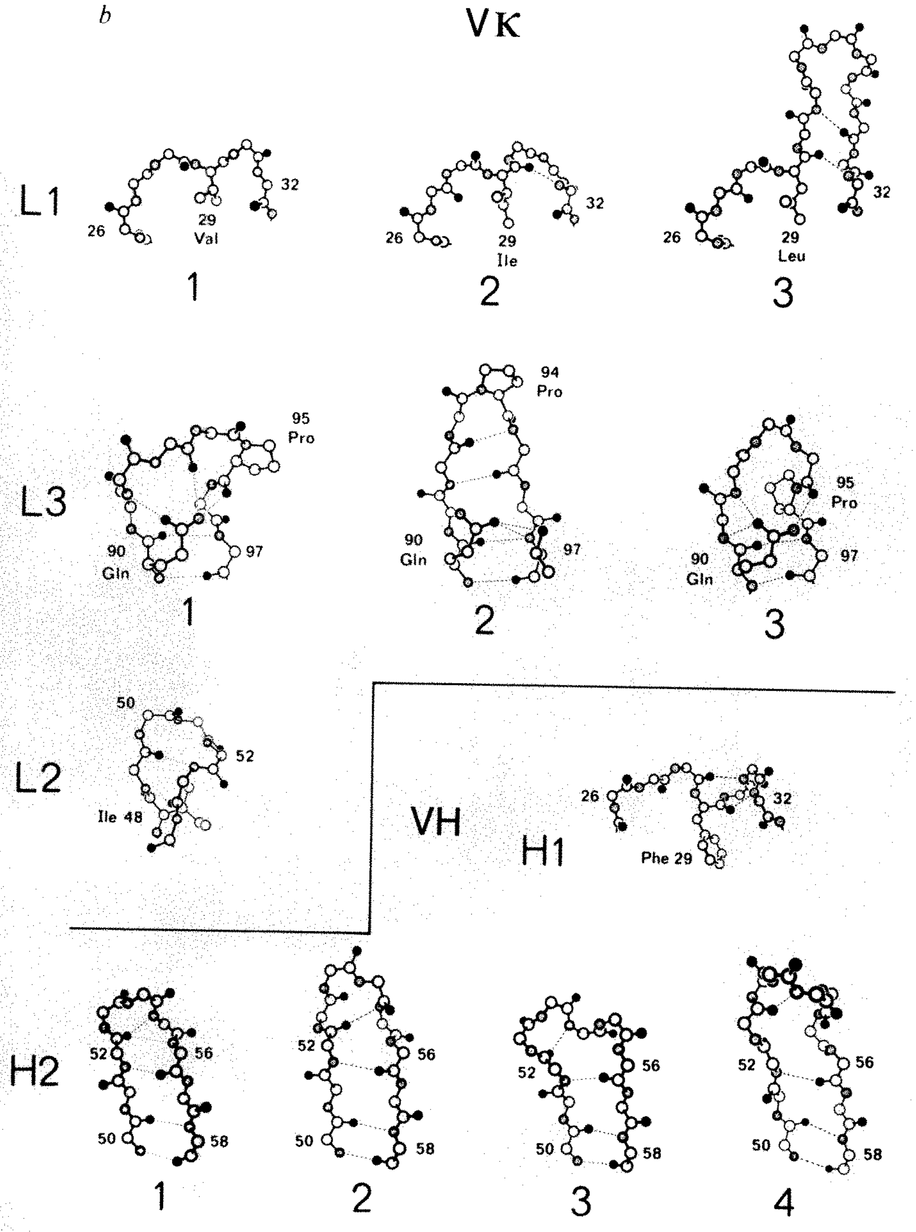


FIG. 1 *a*, Antigen-binding sites of immunoglobulins are formed by six loops of polypeptide, three from the VL domain L1, L2 and L3 and three from the VH domain H1, H2 and H3 (wavy lines). These loops are attached to strands (□) of a conserved β-sheet. *b*, Canonical structures for the hypervariable regions of Vκ and VH domains. In each drawing the region is viewed so that the accessible surface is at top and the framework region below. The main-chain conformation and some of the side chains that determine this conformation are shown. For the definition of the hypervariable regions used here, see Table 1. The immunoglobulins in which the different canonical structures occur are listed in Table 1.

the peptide groups is uncertain. Most side chains are unequivocally placed.

Figure 2 shows the predicted and observed structures of each of the hypervariable regions, superposed by a least-squares fit of their main-chain atoms. Table 2*a* gives, for each predicted and observed hypervariable region, the r.m.s. difference in position of the main-chain atoms.

The main-chain conformations of the predicted and observed hypervariable regions are very similar (Fig. 2, Table 2*a*). The only exception is the H1 region of HyHEL-5. Although the observed and predicted conformations of residues 26-29 and 32 are the same, residues 30 and 31 are in quite different positions. The recently refined structure of Fab J539 (T. N. Bhat, E.A.P. and D.D., manuscript in preparation) shows that these differences were inherited as a result of an error in the original determination of the J539 structure used as the parent for this region. Rebuilding the predicted model with the refined J539 structure puts residues 30 and 31 in the correct position and gives an r.m.s. difference between the predicted and observed H1 regions of 0.6 Å.

Given the medium resolution of the structures used to derive the models and of the experimental structures, the agreement of the predicted and observed loop conformations is excellent.

Relative positions of the hypervariable regions

Figure 3 shows the positions of the hypervariable regions relative to each other and to the framework for the predicted and observed structures. To produce this figure the predicted and observed structures were superposed by a least-squares fit of framework residues. In Table 2*b*, the differences in position of the hypervariable regions are reported.

Small differences in the relative positions of the hypervariable regions in the predicted and observed structures might be expected because of two factors not corrected for in the model building. First, the predicted structures were built using parent V domains that have some residues in the framework and VL-VH interface that are different from those in the final predicted structure. These differences have small but significant effects on the main-chain structure of the individual domains and the way they pack together[2,25,26]. Second, differences between the predicted and observed structures could arise from the effects of the association with the antigen[18]. In other proteins, ligand binding can result in the movement of close-packed segments of polypeptide relative to each other by 1-2 Å, and the ends of loops are able to move somewhat more[27].

The differences in the positions of the predicted and observed H2 regions in HyHEL-5 and NC41 (Table 2*b*) are larger than expected from these factors. The interactions that the H2 regions make with the rest of the VH domain were therefore examined.

Residue 71 and position and conformation of H2

At the same time as the structures of HyHEL-5 and NC41 became available, the refinement of the atomic structure of J539 was completed (T. N. Bhat, E.A.P. and D.D., manuscript in preparation). The conformation of H2 in the refined structure is not like that in HyHEL-5 and NC41 but is the same as that in KOL. This was quite unexpected. The main determinant of the conformation of small turns is usually the position of glycine residues[21]: in KOL, Gly occurs at position 54, and in J539, HyHEL-5 and NC41, Gly occurs at position 55.

An examination of the environments of the H2 regions[28] shows that in KOL and J539 the side chain of framework residue Arg 71 packs between, and forms hydrogen bonds to, H1 and H2. In HyHEL-5, residue 71 is Ala, and here the cavity that would be created by this smaller side chain is filled by the insertion of a residue from the H2 region—Pro 52a. In KOL and J539 the side chain at position 52a is on the surface. The relative movement of position 52a involves a change in the

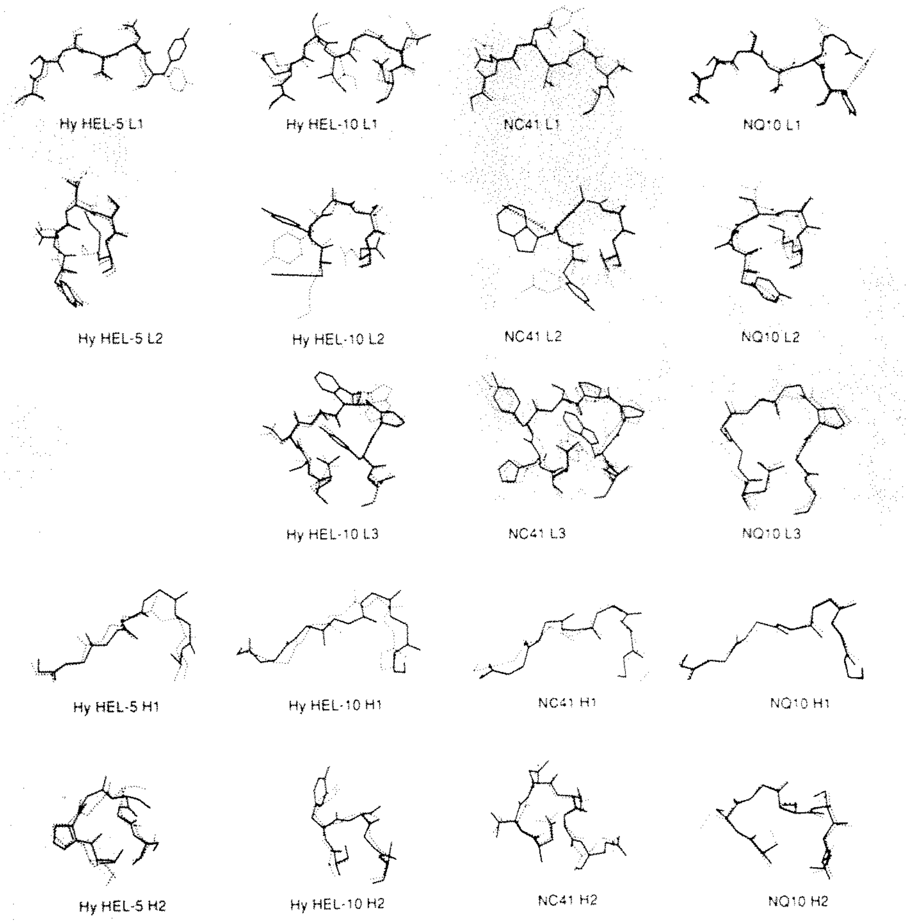


FIG. 2 The predicted (broken line) and observed (continuous line) conformations of the hypervariable regions. The structures have been superposed by a least-squares fit of their main-chain atoms. Residue numbers and the r.m.s. difference in position of the superposed atoms are given in Table 2*a*. Predicted and observed side-chain conformations are shown for all regions except H1 and L3 in NQ10 where they obscure the main chain. After our prediction of the NC41 structure several revisions were made to the sequence and some of the differences can be seen here.

TABLE 2 Differences in structure of predicted and observed hypervariable regions

(*a*) Differences in local conformation (Å)

Hypervariable region	R.m.s. difference in atomic positions of main-chain atoms after optimal superposition			
	HyHEL-5	HyHEL-10	NC41	NQ10
L1:26–32	0.8	1.1	0.7	0.4
L2:49–53	0.9	0.8	0.4	0.9
L3:90–97	—	0.3	0.5	0.6
H1:26–32	1.4	1.3	0.9	0.3
H2:52–56	1.1	1.0	0.7	0.4

(*b*) Differences in position relative to framework (Å)

		Range of differences in positions of C_α atoms after superposition of frameworks residues (Å)	
Protein	Hypervariable region	Original prediction	New structure library
HyHEL-5	L1	2.0–3.8	0.8–2.1
	L2	1.6–1.6	1.2–2.3
	H1	0.8–4.1	0.7–2.1
	H2	3.0–7.2	0.5–2.1
HyHEL-10	L1	0.7–1.6	—
	L2	0.6–.13	—
	L3	0.8–1.5	—
	H1	1.3–3.5	—
	H2	0.6–2.9	—
NC41	L1	1.4–2.4	1.5–2.6
	L2	1.1–1.8	1.0–2.0
	L3	1.6–2.3	1.8–3.0
	H1	1.3–2.0	1.0–2.1
	H2	2.1–4.4	0.4–1.9
NQ10	L1	0.4–2.7	—
	L2	0.5–1.4	—
	L3	0.6–1.5	—
	H1	0.6–1.2	—
	H2	0.6–0.9	—

Superposition of *a* are illustrated in Fig. 2. The differences in the positions of the hypervariable regions in the original predictions and the observed structures of *b* are shown in Fig. 3. The predictions with a new structure library involve rebuilding the HyHEL-5 model using the refined VL J539 and the VH NC41 structures, and rebuilding the NC41 model using VL McPC603 and VH HyHEL-5: see text.

conformation of H2. It also tilts the H2 loop so that the positions of residues at the top of the loop in HyHEL-5 differ in position, relative to those in KOL and J539, by ~4.5 Å. In NC41, the residue at position 71 is Leu and that at position 52a is Thr, and the shift is not as large as that in HyHEL-5.

This analysis implies that the conformation and position of four residue H2 regions are determined by the packing against the VH framework and by the identity of the residue at position 71 in particular, and not by the position in the sequence of H2 of Gly (or Asn or Asp) as was believed previously[8]. Thus VH domains of HyHEL-5 and NC41 should provide better parents for each other than the other structures do, for they contain similar determinants for the position of H2. The predicted structure of HyHEL-5 using the observed structure of VH of NC41 and the predicted structure of NC41 using the observed structure of VH of HyHEL-5 were thus rebuilt. In these new predicted structures the large differences between the predicted and observed positions of H2 are not present: most residues differ by no more than 2.1 Å and none differs by more than 3.0 Å (Table 2*b*).

The results of this analysis were used in the prediction of the structure of the H2 region of the antibody NQ10.

Hypervariable regions of NQ10 and D1.3

The amino-acid sequence of the hypervariable regions and associated framework sites of NQ10 are given in Table 1. For five of the hypervariable regions, the size and the residue conservation at the relevant sites clearly indicate particular canonical structures, and a model of the VL–VH dimer of NQ10 was made using the procedure described above. For H2, after the analysis described above, canonical structure 2 was expecte because of the Arg at position 71 (Table 1).

Recently a crystal structure has been determined for NQ1 (S.S., P.M.A. and R.J.P., manuscript in preparation). This struc ture is determined to a resolution of 2.8 Å and the presen residual is 21%. The tracing of the chain in the hypervariabl regions is unambiguous. Figure 2 shows the predicted an observed structures of each of the hypervariable regions, supe posed by a least-squares fit of their main-chain atoms. Table 2 gives the r.m.s. differences in position of the main-chain atom Figure 3 shows the relative positions of the predicted an observed hypervariable regions.

There is close agreement between the predicted and observe main-chain conformations of the hypervariable regions: th r.m.s. differences in position are between 0.3 and 0.9 Å (Tabl 2*a*). There is also close agreement in the relative positions o the hypervariable regions (Fig. 3 and Table 2*b*). No residue differ by more than 2.7 Å in position and all but two are withi 1.7 Å.

Recently, the atomic structure of antibody D1.3 complexe with lysozyme has been refined[20]. The comparison of the pre liminary experimental structure with the prediction had show differences for two hypervariable regions[9]. The L3 regions ha differences associated with Pro 95 having a *cis*-peptide in th predicted structure and a *trans*-peptide in the observed. The H regions differed because the predicted structure had this regio folded into framework, whereas in the observed it folded ou into the solvent. The refinement of the experimental structur has resulted in the rebuilding of these two regions[20], and thei conformations now agree with the original predictions.

Canonical structures in immunoglobulin 4-4-20

The atomic structure of immunoglobulin 4-4-20 has recentl been determined at a resolution of 2.7 Å (ref. 19). The amino acid sequence of the hypervariable regions and associate framework sites of 4-4-20 are given in Table 1. Four of th hypervariable regions, L2, L3, H1 and H2, have the size an the residues at specific sites that clearly indicate particula canonical structures (see Table 1). These four hypervariabl regions do have the expected main-chain conformations. In Fig 4 the four hypervariable regions, superposed on example of the same canonical structures taken from other immuno globulins, are shown. The r.m.s. difference in the atomi

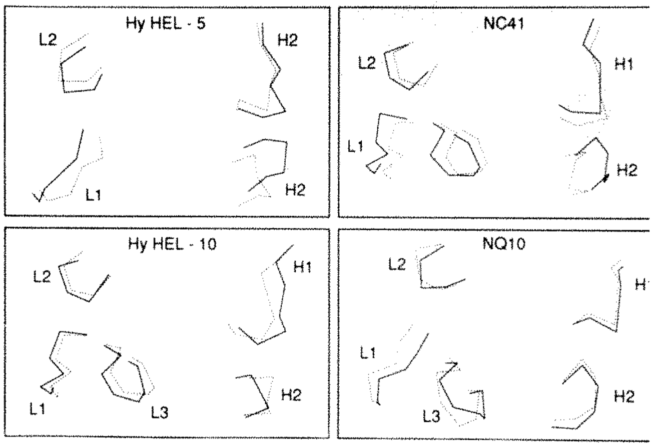


FIG. 3 The relative positions of the hypervariable regions in the predicte and observed structures. The positions of the hypervariable regions show are those given by the superposition of the VL–VH framework regions o the observed and predicted structures. The C_α atoms of residues in th observed structure are joined by solid lines and those in the predicte structure by broken lines. The size of the differences in position are give in Table 2*b*.

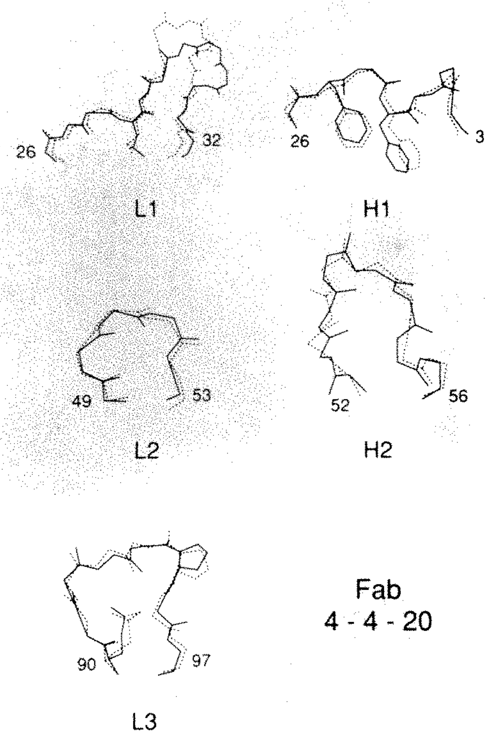


FIG. 4 The hypervariable regions in immunoglobulin 4-4-20. The observed conformations of the hypervariable regions in 4-4-20 are drawn with continuous lines. They are superposed on hypervariable regions from other immunoglobulins that have the same canonical structure—L2 and L3 from REI, H1 from KOL and H2 from McPC603—or in the case of L1, the closely related canonical structure from McPC603. The side chains shown are those that are important in determining the conformation of the region.

position of the main-chain atoms of these pairs are in the range 0.5–0.9 Å.

The L1 region of 4-4-20 has a different size to those found previously and represents a new canonical structure (see Table 1 and Fig. 4). As predicted in a previous discussion of the sequence patterns of this region[8], its conformation is closely related to those of the other canonical structures known for this region. The main-chain atoms of residues 26–31 and 31e–32 fit the same region in McPC603 with a r.m.s. difference in position of 0.8 Å (Fig. 4).

Common occurrence of known canonical structures

The close agreement of the predicted and observed main-chain conformations of the hypervariable regions strongly supports the canonical structure model. It implies that a good estimate can be made of the extent to which hypervariable regions in other immunoglobulins have main-chain conformations close to those that are now known. This can be done by inspecting the known sequences to see whether their hypervariable regions correspond in size to the known canonical structures, and whether they contain one of the sets of residues that produce the observed conformations.

In Table 1 we give the results of examining the immunoglobulin sequences collected by Kabat *et al.*[29]. About 90% of the hypervariable regions in Vκ domains, and ~70% of the H1 and H2 regions in VH domains, are expected to have conformations close to those found in the immunoglobulin structures now known. These estimates are conservative in that they include only hypervariable regions that match the sequence requirements exactly, that is, those that contain the particular residues, or any combination of the small range of closely related residues, that are found in the known structures at the sites marked by asterisks in Table 1.

Discussion

The wide occurrence of the known canonical structures, the use of the much larger library of parent structures that will soon become available, and the more detailed understanding of the determinants of structure that will emerge from the analysis of the differences between predicted and observed structures, should allow, for many immunoglobulins, the prediction of the structure of five of the hypervariable regions with accurate local conformation and errors in position of 2 Å or less.

Predictions of the structures of the H3 regions in the immunoglobulins discussed here could not be made on the basis of the previously known structures. It was possible to predict correctly the H3 conformation in immunoglobulin D1.3 (ref. 9). In general, however, the various genetic mechanisms that produce the H3 regions usually result in medium or large surface loops with very different sequences and patterns of interaction. For such hypervariable regions it may be possible to predict their structure using the conformational search algorithms that have been developed by several groups[30-35].

The results presented here have interesting implications for the molecular mechanisms involved in the generation of antibody diversity. For at least five of the six hypervariable regions of most immunoglobulins there seems to be only a small repertoire of main chain conformations, most of which are known from the set of immunoglobulin structures so far determined. Sequence variations within the hypervariable region modulate the surface that these canonical structures present to the antigens. Sequence variations within the framework and hypervariable regions shift the canonical structures relative to each other by small but significant amounts. □

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1. Alzari, P. M., Lascombe, M.-B. & Poljak, R. J. *A. Rev. Immun.* **6,** 555–580 (1988).
2. Davies, D. R. & Metzger, H *A. Rev. Immun.* **1,** 87–117 (1983).
3. Wu, T. T. & Kabat, E. A. *J. exp. Med.* **132,** 211–250 (1970).
4. Jones, P. T., Dear, P. H., Foote, J., Neuberger, M. S. & Winter, G. *Nature* **321,** 522–525 (1986).
5. Kabat, E. A., Wu, T. T. & Bilofsky, H. *J. biol. Chem.* **252,** 6609–6616 (1977).
6. Padlan, E. A. & Davies, D. R. *Proc. natn. Acad. Sci. U.S.A.* **72,** 819–823 (1975).
7. de la Paz, P., Sutton, B. J., Darsley, M. J. & Rees, A. R. *EMBO J.* **5,** 415–425 (1986).
8. Chothia, C. & Lesk, A. M. *J. molec. Biol.* **196,** 901–917 (1987).
9. Chothia, C. *et al. Science* **233,** 755–758 (1986).
10. Poljak, R. J., Amzel, L. M., Chen, B. L., Phizackerley, R. P. & Saul, F. *Proc. natn. Acad. Sci. U.S.A.* **71,** 3440–3444 (1974).
11. Satow, Y., Cohen, G. H., Padlan, E. A. & Davies, D. R. *J. molec. Biol.* **190,** 593–604 (1986).
12. Marquart, M., Deisenhofer, J. & Huber, R. *J. molec. Biol.* **141,** 369–391 (1980).
13. Suh, S. E. *et al. Proteins* **1,** 74–80 (1986).
14. Epp, O., Latham, E., Schiffer, M., Huber, R. & Palm, W. *Biochemistry* **14,** 4943–4952 (1975).
15. Furey, W., Wang, B. C., Yoo, C. S. & Sax, M. *J. molec. Biol* **167,** 661–692 (1983).
16. Sheriff, S. *et al. Proc. natn. Acad. Sci. U.S.A.* **84,** 8075–8079 (1987).
17. Padlan, E. A. *et al. Proc. natn. Acad. Sci. U.S.A.* **86,** 5938–5942 (1989).
18. Colman, P. M. *et al. Nature* **326,** 358–362 (1987).
19. Herron, J. N., He, X.-m., Mason, M. I., Voss, E. W. & Edmundson, A. B. *Proteins* **5,** 271–286 (1989).
20. Bentley, G. A. *et al. Cold Spring Harb. Symp. quant. Biol.* Vol. LIV (in the press).
21. Sibanda, B. L., Blundell, T. L. & Thornton, J. M. *J. molec. Biol.* **206,** 759–777 (1989).
22. Lesk, A. M. & Chothia, C. H. *Phil. Trans. R. Soc.* A**317,** 345–356 (1986).
23. Summers, N. L., Carlson, W. D. & Karplus M. *J. molec. Biol.* **196,** 175–198 (1987).
24. Levitt, M. J. *Molec. Biol.* **170,** 723–764 (1974).
25. Chothia, C. & Lesk, A. M. *EMBO J.* **5,** 823–826 (1986).
26. Lascombe, M.-B. *et al. Proc. natn. Acad. Sci. U.S.A.* **86,** 607–611 (1989).
27. Chothia, C. & Lesk, A. M. *Trends. Biochem. Sci.* **10,** 116–118 (1985).
28. Tramontano, A., Chothia, C. & Lesk, A. M. (submitted).
29. Kabat, E. A., Wu, T. T., Reid-Miller, M., Perry, H. M. & Gottesman, K. S. *Sequences of Proteins of Immunological Interest* 4th edn (Public Health Service, NIH, Washington DC, 1987).
30. Moult, J. & James, M. N. G. *Proteins* **1,** 146–163 (1986).
31. Snow, M. E. & Amzel, L. M. *Proteins* **1,** 267–279 (1986).
32. Fine, R. M., Wang, H., Shenkin, P. S., Yarmush, D. L. & Levinthal, C. *Proteins* **1,** 342–362 (1986).
33. Bruccoleri, R. E. & Karplus, M. *Biopolymers* **26,** 137–168 (1987).
34. Bruccoleri, R. E., Haber, E. & Novotny, J. *Nature* **335,** 564–568 (1988).
35. Martin, A. C. R., Cheetham, J. C. & Rees, A. R. *Proc. natn. Acad. Sci. U.S.A.* (in the press).

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Reconstitution of active dimeric ribulose bisphosphate carboxylase from an unfolded state depends on two chaperonin proteins and Mg-ATP

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In vitro reconstitution of active ribulose bisphosphate carboxylase (Rubisco) from unfolded polypeptides is facilitated by the molecular chaperones: chaperonin-60 from *Escherichia coli* (groEL), yeast mitochondria (hsp60) or chloroplasts (Rubisco subunit-binding protein), together with chaperonin-10 from *E. coli* (groES), and Mg-ATP. Because chaperonins are ubiquitous, a conserved Mg-ATP-dependent mechanism exists that uses the chaperonins to facilitate the folding of some other proteins.

THE chaperonins[1] are a ubiquitous class of proteins implicated in the post-translational folding and assembly of other proteins. They include the groEL or chaperonin-60 (cpn60) and the groES or chaperonin-10 (cpn10) proteins from bacteria[2-4], chloroplasts[5] and mitochondria[6-8]. The earliest evidence for the involvement of the chaperonins with post-translational events was the demonstration that the *E. coli* cpn60 and cpn10 proteins are essential for phage head morphogenesis[9-11]. The chloroplast version of cpn60 is the Rubisco subunit-binding protein, so-called because of its transient association with newly synthesized Rubisco subunits[12-14]. The transient association of newly synthesized unfolded proteins with the chaperonins has also been observed during the formation of pre-beta-lactamase and chloramphenicol acetyltransferase[15] in *E. coli*, during the recombinant expression in *E. coli* of the precursor to the small subunit of Rubisco[16], and also after the import of precursor proteins into yeast mitochondria[17,18] and chloroplasts[19]. But the molecular details of what happens while these polypeptides are associated with the chaperonins remains to be determined.

Recently, we demonstrated *in vivo* that the *E. coli* chaperonins cpn60 and cpn10 promoted the post-translational folding or assembly, or both, of hexadecameric (L_8S_8) and dimeric (L_2) Rubiscos in *E. coli*[20]. We suggested that the chaperonins exert their effect post-translationally, at some point between the unfolded nascent monomer (L) and the formation of the folded dimer (L_2), the basic structural unit of the more complex hexadecamer[21-23]. We have now used the purified *E. coli* chaperonins cpn60 and cpn10 to demonstrate that the *in vitro* reconstitution of the catalytically functional dimeric form of Rubisco from an unfolded biologically inert polypeptide depends on the presence of both chaperonin proteins and Mg-ATP.

The substrate—denatured Rubisco

Rubisco from *Rhodospirillum rubrum* is a dimer of large subunits only[24]. Genetic[25] and structural[21] studies have established that the dimer is the minimal structural entity capable of catalysis, a single active site being composed of amino-acid residues from both subunits.

Because our genetic analysis[20] indicated that the chaperonins functioned post-translationally at some point before the formation of the folded functional dimer, we sought conditions for unfolding native Rubisco in the belief that unfolded Rubisco would serve as a substrate for the chaperonins. To determine the change in secondary structure accompanying the various denaturing regimes, we recorded circular dichroism (CD) spectra in the far ultraviolet range. The spectrum for native decarbamylated Rubisco (Fig. 1, E) showed negative molar ellipticity ($\theta_{220} = -10^4$ degrees · cm^2 · dmol^{-1}) that is expected for a protein with this structure. On denaturation with either 8 M

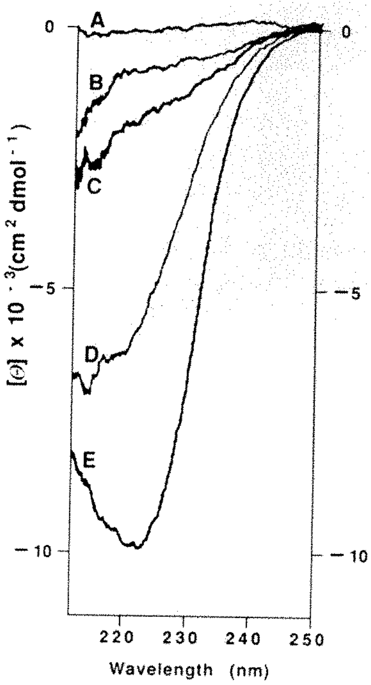

FIG. 1 CD spectra of native and denatured Rubiscos. A, baseline; B, denatured Rubisco (Rubisco-U) in 6 M guanidine-HCl, 0.1 M Tris-HCl buffer, pH 7.85; C, denatured Rubisco (Rubisco-U) in 8 M urea, 0.1 M Tris-HCl buffer pH 7.85; D, denatured Rubisco (Rubisco-A) in 0.1 M glycine-HCl, pH 3.0; and E, native decarbamylated Rubisco in 0.1 M Tris-HCl buffer, pH 7.85. Concentration of Rubisco protomer, 7.9 μM; temperature, 25 °C.

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urea (Fig. 1, C) or 6 M guanidine-HCl (Fig. 1, B), the negative ellipticity was strongly reduced indicating a considerable loss of secondary structure. We conclude that treatment with either 8 M urea or 6 M guanidine-HCl converts Rubisco into unfolded monomeric polypeptides. We refer to Rubisco in this state as Rubisco-U.

Treatment of Rubisco with acid (pH 3) led to a rapid ($t_{1/2} \sim$ 30 s) loss of activity which was not recovered on neutralization. The CD spectrum of acid-denatured Rubisco (Fig. 1, D) showed some reduction of the negative band ($\theta_{220} = -6 \times 10^3$ degrees·cm^2·dmol^{-1}). It is evident, however, that much secondary structure remains under these conditions. Whether or not the structure of the acid-denatured Rubisco corresponds to the molten-globule or A-state, observed with other proteins[26-28] at acidic pH, requires further investigation. We refer to acid-denatured Rubisco in this partly unfolded state as Rubisco-A.

Chaperonin-dependent Rubisco reconstitution

Attempts to spontaneously reconstitute active Rubisco from both Rubisco-U and Rubisco-A under various conditions were unsuccessful. By contrast, when either Rubisco-U or Rubisco-A was diluted into solutions containing both chaperonins cpn60 and cpn10, and Mg-ATP, Rubisco activity, indicative of a folded dimer, was reconstituted (Fig. 2*a*). No reconstitution occurred when cpn60 or cpn10 was omitted. The time course of reconstitution was followed by using a glucose-hexokinase quench, demonstrating the requirement for Mg-ATP (Fig. 2*a*, *b*). The maximum reconstitution was ~80%. Rubisco-U consistently yielded the greatest quantity of reconstituted enzyme. Rubisco-A was reconstituted to ~40% (Fig. 2*a*). Although the extent of reconstitution varied, depending on whether Rubisco-U or Rubisco-A was used as substrate, the rate constant for the chaperonin-dependent reconstitution was the same, within the limit of experimental error. This implies that reconstitution involves a common intermediate, the formation of which preceeds the same chaperonin-dependent rate-determining step. We propose that on removal of the denaturant by dilution, a rapid chaperonin-independent folding of Rubisco-U to a state rich in secondary structure and resembling Rubisco-A (Rubisco-I state), is followed by reaction with cpn60, cpn10 and Mg-ATP.

Reconstitution of Rubisco involves two processes. Folding of the polypeptide, which is independent of concentration, followed by the assembly of the folded but inactive monomers into catalytically competent dimers. Assembly depends on the concentration of monomers and on the dissociation constant governing their interaction. The magnitude of this dissociation constant is so far unknown. We anticipated that the appearance of Rubisco activity might, however, be preceded by a lag period, during which sufficient folded monomers accumulate before dimerization. Inspection of the earliest part of the reconstitution (Fig. 2*b*) revealed just such a lag. We estimate that detectable dimer formation requires the accumulation of ~2 nM folded monomers. A similar value was computed after the synthesis of Rubisco in a prokaryotic cell-free transcription–translation system[29].

The chaperonin-dependent reconstitution system was examined as a function of the concentrations of Rubisco, cpn60 and cpn10, varying the concentration of one component at a time (Fig. 3). First, no reconstitution occurred when either cpn10 (Fig. 3*a*) or cpn60 (Fig. 3*b*) was omitted. Second, optimal reconstitution occurred when cpn60 and cpn10 were approximately equimolar (Fig. 3*a*, *b*). Note, however, that reconstitution was inhibited when the concentration of cpn60 exceeded that of cpn10 (Fig. 3*b*).

The dependency of the reconstitution system on the Rubisco concentration was more complex (Fig. 3*c*) and reflected the interplay of two processes. At the lowest concentration of Rubisco used (3 nM protomer), only 20% reconstitution occurred. But the yield of active enzyme increased with increas-

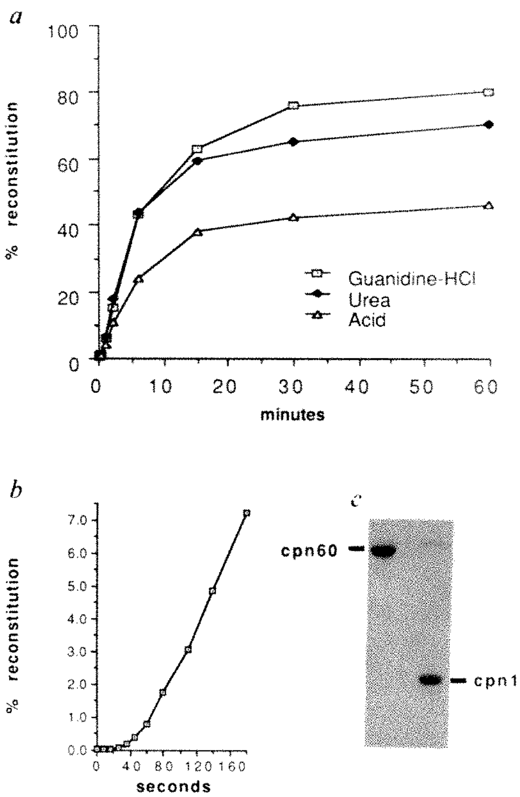


FIG. 2 The time-course of chaperonin- and Mg-ATP-dependent reconstitution of active Rubisco from variously denatured Rubiscos (Rubisco-U or Rubisco-A). *a*, Complete time courses of reconstitution. *b*, Lag in the formation of active Rubisco dimers from a different experiment to that represented in *a*. In both cases Rubisco activity is expressed as a percentage of the activity of an equal quantity of native non-denatured enzyme. *c*, SDS-PAGE of the purified preparations of cpn60 and cpn10 from *E. coli* used in this study. METHODS. The *E. coli* chaperonins cpn60 and cpn10 were purified from lysates of cells bearing the multicopy plasmid pGroESL[20], by modifications (to be described elsewhere) of published purification protocols[30,38]. Recombinant dimeric Rubisco was purified from *E. coli* expressing a plasmid-encoded gene from *R. rubrum* as previously described[39,40]. *a*, Rubisco-U was prepared by incubating 15.4 μM Rubisco (protomer) in 0.1 M Tris–HCl buffer, 0.01 M EDTA, pH 7.7, containing either 5.7 M guanidine-HCl or 7.6 M urea for 2 h at 25 °C. Rubisco-A was prepared by incubating 15.4 μM Rubisco (protomer) in 50 mM glycine-HCl, pH 3.0, for 210 s at 25 °C. Reconstitution was initiated by diluting 6.7 μl of the denatured Rubisco-U or Rubisco-A into 1,418 μl solution containing 50 mM Tris–HCl buffer, pH 7.5, 2 mM ATP, 12 mM $MgCl_2$, 10 mM glucose, 4.3 μM cpn60 (protomer) and 9.6 μM cpn10 (protomer) at 25 °C. The final concentration of Rubisco protomer in the reconstitution reaction was 72 nM. At the times indicated a 150 μl aliquot was withdrawn and added to 10 μl solution containing 6 U of hexokinase so as to arrest the reconstitution reaction. After the last aliquot had been withdrawn, the reaction mixtures were supplemented with 50 mM $[^{14}C]NaHCO_3$ (300 d.p.m. nmol^{-1}) and allowed to activate for a further 5 min. Ribulose bisphosphate (1 mM) was added and Rubisco activity measured as the formation of acid-stable ^{14}C over 30 min at 25 °C. The activity of native Rubisco (100%) under these conditions was 2.7 s^{-1}. *b*, Rubisco-U (20.4 μM protomer) was prepared in guanidine-HCl as described above. Reconstitution was initiated by diluting 21 μl Rubisco-U into 1,659 μl solution containing 50 mM Tris–HCl buffer, pH 8.0, 3 mM ATP, 7 mM $MgCl_2$, 20 mM glucose, 4 μM cpn60 (protomer) and 6 μM cpn10 (protomer) at 23 °C. At the times indicated, the reconstitution reaction was quenched with hexokinase and Rubisco activity determined as described above. *c*, Samples of purified cpn60 and cpn10 containing ~4 μg were boiled in 1% SDS for 5 min and applied to a 16% SDS-polyacrylamide gel. After electrophoresis, the gel was stained with Coomassie blue.

ing concentration until an optimum (for this particular concentration of chaperonins) was reached at ~50 nM Rubisco. These results are again consistent with the need to accumulate sufficient folded monomers to drive the assembly of active dimers. Optimal reconstitution was reached at a cpn60 : Rubisco-protomer concentration ratio of ~50:1 (Fig. 3*c*). But because reconstitution involved the oligomeric form of cpn60, the double-doughnut-shaped 14-mer[30,31] ($[\text{cpn60}]_{14}$) (Fig. 4, B and C), the molar ratio of $[\text{cpn60}]_{14}$ to Rubisco protomers was closer to 3:1. The requirement for excess cpn60 over Rubisco was largely due to the instability of the partly folded intermediate, Rubisco-I, that is believed to be generated from both Rubisco-U and Rubisco-A on removal of the denaturant, and its propensity to irreversibly aggregate (Fig. 4). The decline in the yield of reconstituted Rubisco observed as the concentration of Rubisco-U was increased was due to irreversible aggregation (Fig. 3*c*). Thus, when either Rubisco-U or Rubisco-A was diluted into solutions containing the chaperonins, two competitive and mutually exclusive processes occurred. The unfolded or partly folded protomers either formed biologically unproductive aggregates or they formed a binary complex with $[\text{cpn60}]_{14}$ (see below), which prevented aggregation and directed the protomers along a biologically productive pathway.

Homologous cpn60s also support reconstitution

The prokaryotic cpn60 is homologous to the eukaryotic chaperonins hsp60, from yeast mitochondria, and the Rubisco subunit-binding protein, from plant chloroplasts[5,7]. We purified both proteins to homogeneity and used them in a heterologous reconstitution system with *E. coli* cpn10. We used approximately equal concentrations of each of the cpn60 proteins together with equal concentrations of cpn10. The heterologous chaperonin combinations cpn60 (yeast)-cpn10 (*E. coli*), and cpn60 (chloroplast)-cpn10 (*E. coli*) were respectively 10% and 25% as effective as the homologous cpn60–cpn10 (*E. coli*) combination in facilitating the reconstitution of Rubisco-U. This was not unexpected, because the chaperonins of a given organism have presumably evolved to function together.

Cpn60 traps an unstable folding intermediate

Evidence that Rubisco-U is rapidly converted to an unstable intermediate state, Rubisco-I, on removal of the denaturant, and the ability of cpn60 to stabilize this intermediate was provided by the delay experiment, the results of which are shown in Fig. 4. In this experiment, Rubisco-U was diluted into a solution lacking cpn60. Cpn60 was added 5–60 s later. Cpn10 was added ~10 min later to complete the reconstitution mixture. The ability of the chaperonins to reconstitute activity from Rubisco-U was rapidly lost as the time between the dilution of Rubisco-U and the addition of cpn60 increased. The rate at which recoverable activity was lost increased as the concentration of Rubisco protomers increased (Fig. 4), indicating that an unstable folding intermediate—Rubisco-I, formed from Rubisco-U on dilution—was susceptible to irreversible aggregation. This accounts for the progressive decline in the yield of reconstituted Rubisco observed as the concentration of Rubisco-U introduced into the system was increased (Fig. 3*c*). We conclude that when Rubisco-U is diluted into solutions containing the chaperonins, two competitive and mutually exclusive processes occur—aggregation or formation of a binary complex with $[\text{cpn60}]_{14}$.

Rubisco-I–$[\text{cpn60}]_{14}$ binary complex is stable

We demonstrated the physical existence of a stable binary complex between Rubisco-I and cpn60, and the conditions leading to the formation of an active dimer of Rubisco, by immunoelectrophoretic procedures (Fig. 5). When Rubisco-U was diluted into a solution containing cpn60, cpn10 and Mg-ATP, Rubisco activity, indicative of folded dimers, was reconstituted (Fig. 5*a*, column 7). The dimer was detected immunochemically after non-denaturing PAGE (Fig. 5*b*, track 7). When one or more of the components was omitted, or when the chaperonins were first boiled, no reconstitution of Rubisco activity occurred (Fig. 5*a*, columns 2–6, 8 and 9, respectively) and no cross-reactive band corresponding to the dimer was detected (Fig. 5*b*, tracks 2–6, and 8 and 9, respectively). In the presence of native cpn60, but when either cpn10 or Mg-ATP or both were absent,

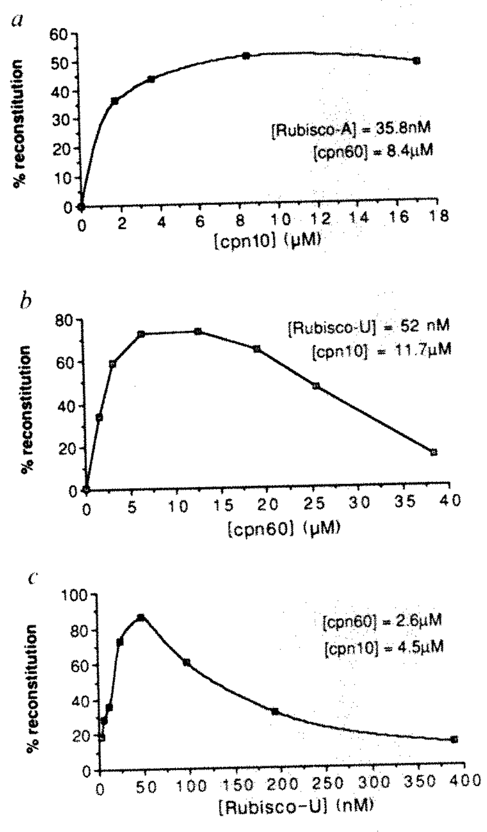


FIG. 3 The chaperonin- and Mg-ATP-dependent reconstitution of active Rubisco as a function of the concentrations of Rubisco, cpn60 and cpn10. The yield of reconstituted Rubisco as function of variable cpn10 concentration, as indicated, constant cpn60 concentration, constant Rubisco concentration (*a*); variable cpn60 concentration as indicated, constant cpn10 concentration, constant Rubisco concentration (*b*); variable Rubisco concentration as indicated, constant cpn60 concentration, constant cpn10 concentration (*c*). Note that these are not measurements of the initial rates of reconstitution, but the extent of complete reconstitution after 1–3 h.

METHODS. *a*, Rubisco-A was prepared by incubating 1.8 mM Rubisco (protomer) in 50 mM glycine-HCl, pH 3.0, for 4 min at 23 °C. Reconstitution was initiated by diluting 5 μl Rubisco-A into 245 μl solution containing 75 mM Tris–HCl buffer, pH 7.7, 7.5 mM $MgCl_2$, 5 mM ATP, 1 mg ml^{-1} BSA, 8.4 μM cpn60 (protomer) and various concentrations (0–17.8 μM protomer) of cpn10. A control containing an equal quantity of native Rubisco was also prepared. After incubation for 1 h at 23 °C, Rubisco activity was determined as described in the legend to Fig. 2. *b*, Rubisco-U (3.9 μM protomer) was prepared as described above. Reconstitution was initiated by diluting 4 μl Rubisco-U (3.9 μM) into 296 μl solution containing 11.7 μM cpn10 (protomer), 50 mM Tris–HCl buffer, pH 8.0, 15 mM $MgCl_2$, 4 mM ATP and various concentrations (0–38.4 μM protomer) of cpn60. Controls containing an equal quantity of native Rubisco were prepared. After incubation at 25 °C for 1 h, Rubisco activity was determined as described in the legend to Fig. 2. *c*, Various concentrations (0.31 to 39 μM protomer) of denatured Rubisco-U were prepared by incubating the appropriate amount of Rubisco in 5.7 M guanidine-HCl, 0.1 M Tris–HCl buffer, pH 7.7, 0.01 M EDTA for 1 h at 23 °C. Reconstitution was initiated by diluting 2.5 μl Rubisco-U into 247.5 μl solution containing 2.6 μM cpn60 (protomer), 4.5 μM cpn10 (protomer), 2 mM ATP, 12.5 mM $MgCl_2$ and 75 mM Tris–HCl buffer, pH 7.8. Controls using the same quantity of native Rubisco were prepared. After incubation for 3 h at 25 °C, Rubisco activity was determined as in the legend to Fig. 2, with the exception that the duration of the Rubisco assay was varied according to the quantity of enzyme present.

two electrophoretically distinct immunochemically cross-reactive signals were observed (Fig. 5*b*, tracks 3, 4 and 6). The first sharply defined band, revealed with antibody against Rubisco, corresponded to the binary complex of Rubisco subunits bound to the oligomeric form $[\text{cpn60}]_{14}$. The same oligomer of cpn60, whether or not associated with Rubisco-I, was also revealed with antibody against cpn60 (Fig. 5*c*, tracks 1, 3, 4, 6 and 7).

The second cross-reactive signal revealed with antibody against Rubisco, represented a poorly defined smear of Rubisco subunits with a slower electrophoretic mobility than the dimer (Fig. 5*b*, tracks 3, 4 and 6). The nature of these soluble Rubisco subunits is puzzling. They have no catalytic activity. Perhaps they are misfolded or partly folded monomers which arise during electrophoresis by dissociation of the binary complex.

The binary complex could also be demonstrated starting with acid-denatured Rubisco-A (data not shown). Therefore, we conclude that a common folding intermediate, Rubisco-I, is generated from both Rubisco-U and Rubisco-A on dilution, and that Rubisco-I is stabilized by forming a binary complex with $[\text{cpn60}]_{14}$. Because Rubisco-A, and by inference Rubisco-I, has significant secondary structure (Fig. 1), it follows that considerable folding of Rubisco-U to the state represented by Rubisco-I must occur before interaction with cpn60.

The experiment represented in Fig. 5 provided further insight into the mechanism of the chaperonin-dependent reconstitution reaction. First, note that native Rubisco showed no detectable interaction with cpn60 (Fig. 5*b*, *c*; tracks 1 and 10). In the absence of Mg-ATP, cpn60 moved electrophoretically on non-denaturing gels as a single oligomeric species of high relative molecular mass, $[\text{cpn60}]_{14}$ (refs 30 and 32). In the presence of Mg-ATP, however, dissociation of $[\text{cpn60}]_{14}$ to a species of lower relative molecular mass occurred, and a second band, designated $[\text{cpn60}]_n$, appeared on the western blot (Fig. 5*c*, tracks 1, 4, and 7–9). The number of cpn60 subunits in $[\text{cpn60}]_n$ is not known. The chloroplast cpn60, the Rubisco subunit-binding protein, has been reported to behave similarly[13,32]. Although the addition of Mg-ATP to the mixture of the binary complex and uncomplexed $[\text{cpn60}]_{14}$ caused the formation of a smaller species of cpn60, Mg-ATP alone did not lead to the formation of active Rubisco (Fig. 5*a*, column 4), nor to the discharge of the binary complex (Fig. 5*b*, track 4). Similarly, the addition of cpn10 alone led neither to the formation of active Rubisco (Fig. 5*a*, column 6) nor to the discharge of the binary complex (Fig. 5*b*, track 6). Only when both cpn10 and Mg-ATP were added was the binary complex discharged with the

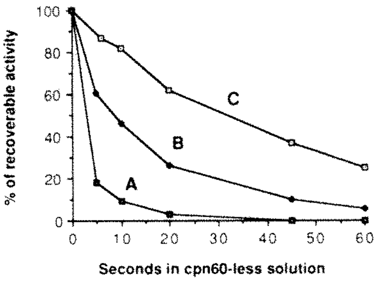
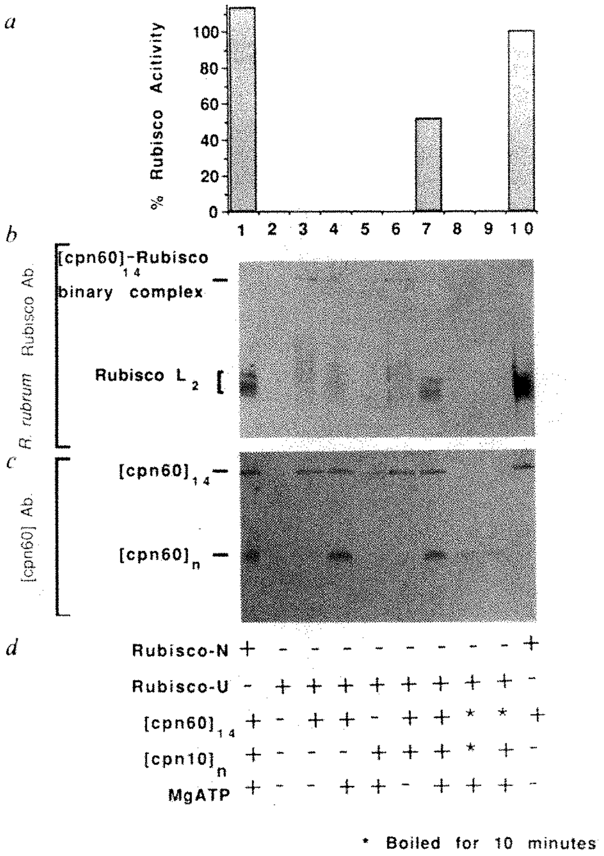


FIG. 4 The instability of the folding intermediate $[\text{Rubisco-I}]_1$ in the absence of cpn60 and the concentration dependence of its decay–aggregation. Rubisco-U 109 nM protomer (A), 54.3 nM protomer (B) or 27.2 nM protomer (C) was incubated in solutions not containing cpn60 (cpn60-less solutions) for the indicated times, when cpn60 was added to stabilize the folding intermediate $[\text{Rubisco-I}]_1$.

METHODS. Rubisco-U at three different concentrations (10.9 μM, 5.4 μM and 2.7 μM protomer) was prepared in 5.7 M guanidine-HCl, 0.1 M Tris-HCl buffer, pH 7.7, 1 mM dithiothreitol and 0.1 mM EDTA. At time zero, 50-μl aliquots of Rubisco-U were diluted into 950 μl solution containing 50 mM Tris-HCl buffer, pH 8.0, 0.5 mg ml^{-1} BSA, 15 mM $MgCl_2$, and 5 mM ATP at 23 °C. At the times indicated, 50-μl aliquots were withdrawn and added to 250 μl solution containing 50 mM Tris-HCl buffer, pH 8.0, 15 mM $MgCl_2$, 5 mM ATP and 6.3 μM cpn60 (protomer) so as to give the following concentrations of Rubisco protomer: 109 nM (A), 54.3 nM (B) and 27.2 nM (C). About 10 min after the last aliquot had been withdrawn, 6 μl cpn10 (protomer; 145 μM) were added to initiate reconstitution. After 1 h at 25 °C, Rubisco activity was determined as described in the legend to Fig. 2. For the time-zero points, 2.5 μl Rubisco-U were diluted directly into 297.5 μl cpn10-less reconstitution mixture. Subsequent treatment was as described above. Controls using equal quantities of native Rubisco were prepared. The data are normalized to the time-zero points for each concentration of Rubisco-U.



Rubisco-N	+	-	-	-	-	-	-	-	-	+
Rubisco-U	-	+	+	+	+	+	+	+	+	-
$[\text{cpn60}]_{14}$	+	-	+	+	-	+	+	*	*	+
$[\text{cpn10}]_n$	+	-	-	-	+	+	+	*	+	-
MgATP	+	-	-	+	+	-	+	+	+	-

* Boiled for 10 minutes

FIG. 5 Chaperonin- and Mg-ATP-dependent reconstitution of active dimeric Rubisco from denatured Rubisco-U. *a*, Rubisco activity, expressed as a percentage of the activity observed with an equal quantity of non-denatured, native Rubisco. *b*, Western blot after non-denaturing PAGE of the reaction mixtures used in *a* and probed with antibody (Ab) raised against Rubisco. The positions of $[\text{cpn60}]_{14}$ and of native Rubisco are indicated. The binary complex of $[\text{cpn60}]_{14}$-Rubisco was not resolved from uncomplexed $[\text{cpn60}]_{14}$. Note the association of Rubisco with $[\text{cpn60}]_{14}$ in tracks 3, 4 and 6. The achromic band in the middle of the native Rubisco bands in tracks 1 and 7 is due to cpn10, which interferes with the interaction of Rubisco and its antibody. *c*, As in *b*, but probed with antibody raised against cpn60. Note the dissociation of $[\text{cpn60}]_{14}$ to a lower relative molecular mass species, $[\text{cpn60}]_n$, in the presence of Mg-ATP. *d*, Summary of the reaction conditions.

METHODS: Denatured Rubisco-U was prepared by incubating 15.4 μM Rubisco (protomer) in 5.7 M guanidine-HCl, 0.1 M Tris-HCl buffer, pH 7.7, 1 mM dithiothreitol and 0.1 mM EDTA for 3 h at 25 °C. Reconstitution was initiated by diluting 2.5 μl above solution into 247.5 μl solution containing (when complete) 50 mM Tris-HCl buffer, pH 7.7, 4.0 mM ATP, 12 mM $MgCl_2$, 1 mM dithiothreitol, 4.0 μM cpn60 (protomer) and 7.6 μM cpn10 (protomer). Individual components were omitted as indicated in *d*. When chaperonins were omitted (as in tracks 2–5 and 10), an equivalent molar concentration of BSA was added. Boiled (100 °C, 10 min) chaperonins were added as indicated (tracks 8 and 9). Controls employing an equal concentration of native Rubisco (tracks 1 and 10) were prepared. Alternatively, after the period for reconstitution, aliquots of the reaction mixtures were analysed by non-denaturing PAGE on 6.5% gels, followed by immunodetection using antibodies raised against either Rubisco (*b*) or cpn60 (*c*). To suppress the nonspecific binding of anti-Rubisco antibodies to cpn60 in *b*, 15 μg/ml^{-1} purified cpn60 was added to the 5% milk-blocking mixture.

appearance of the native dimer (Fig. 5*b*, track 7) and of Rubisco activity (Fig. 5*a*, column 7). From these experiments we conclude that the chaperonin-dependent reconstitution of Rubisco involves a strictly ordered set of reactions—first, the formation of a stable bindary complex of $[\text{cpn60}]_{14}$-$[\text{Rubisco-I}]_1$, which at least *in vitro* can be demonstrated as an Mg-ATP- and cpn10-independent event, followed by the Mg-ATP- and cpn10-dependent discharge of folded and stable, but catalytically inactive, monomers, which subsequently assemble into active dimers.

Cpn10 and ATP react with the binary complex

We determined the initial rate of reconstitution as a function of the concentration of Mg-ATP. On this basis the Michaelis constant K_m for Mg-ATP was ~0.3 mM (data not shown). Other nucleotide triphosphates (CTP, GTP, UTP and dATP) were investigated for their ability to support reconstitution. Rubisco activity was determined after one hour's incubation in the presence of cpn60, cpn10 and 5 mM nucleotide triphosphate. No reconstitution was seen with GTP, UTP and dATP. But CTP supported a chaperonin-dependent reconstitution of Rubisco activity, with a yield of ~50%. Non-hydrolysable analogues of ATP (α,β-methylene adenosine 5′-triphosphate, β,γ-methylene adenosine 5′-triphosphate and β,γ-imidoadenosine-5′-triphosphate) did not support reconstitution. Nor did adenosine 5′-(γ-thio)triphosphate support reconstitution. Furthermore, adenosine 5′-(γ-thio) triphosphate inhibited the ATP-dependent reconstitution reaction (data not shown), indicating that at least this ATP-analogue can bind to the chaperonin protein. The failure of these ATP analogues to support the chaperonin-dependent reconstitution of Rubisco indicates that the hydrolysis of ATP could be involved.

Discussion

The essential function of the chaperone proteins, as proposed by Pelham[33], and by Ellis and colleagues[1,34], is to facilitate the folding of other polypeptides and their assembly into oligomeric structures of which they are not a part. It was further proposed that molecular chaperones both prevent the formation of incorrect structures and unscramble those that do occur. The experiments reported here provide experimental evidence both for and against the three elements of this hypothesis. First, the *E. coli* chaperonins cpn60 and cpn10 facilitate a Mg-ATP-dependent conversion of an unfolded or partly folded polypeptide—Rubisco in this instance—into a correctly folded catalytically active dimer, in accordance with the main postulate. Second, the folding of Rubisco involves an unstable intermediate, Rubisco-I, with a propensity to aggregate to form biologically unproductive structures. By forming a stable binary complex with cpn60, the formation of these incorrect structures is prevented and the folding intermediate directed down a biologically productive pathway. But the chaperonins are unable to rescue Rubisco molecules which aggregate. This step seems irreversible, at least on the timescale of our experiments.

The failure of both Rubisco-U and Rubisco-A to fold spontaneously under the conditions we have used and within the time frame of the experiments described here does not exclude the possibility that they could be induced to do so under a different set of conditions. But regardless of that, we are left with the fact that the chaperonins enhance the rate of reconstitution of Rubisco under the chosen conditions by a very substantial factor. Therefore, along with *cis-trans* prolyl isomerase[35,36] and protein disulphide isomerase[37], the chaperonins accelerate the rate of folding of other proteins. Unlike *cis-trans* prolyl isomerase and protein disulphide isomerase, whose molecular mechanisms are understood in principle, if not in detail, however, the molecular basis for the rate enhancement observed with the chaperonins is at the moment obscure.

The chaperonin-facilitated reconstitution of Rubisco activity involves two steps—a folding step, followed by the assembly of two folded but catalytically inactive monomers into a catalytically active dimer. The results presented here are consistent with the notion that the chaperonins facilitate at least some part of the folding step, with assembly of the dimer occurring subsequently and independently of the chaperonins.

The requirement for a large molar excess of chaperonin over the substrate Rubisco-U or Rubisco-A (Fig. 3) is largely due to the unstable nature of the folding intermediate Rubisco-I, generated on diluting Rubisco-U or Rubisco-A into the reaction solution. It is clear from Fig. 4 that the concept of a stable substrate 'patiently' awaiting interaction with sub-stoichiometric quantities of a catalyst does not apply here. Instead, there is an urgent need to stabilize this fickle intermediate by formation of a binary complex with cpn60. A large excess of cpn60 assures the formation of the stable biologically productive binary complex. The need to sequester unstable folding intermediates evidently exists *in vivo* as well as *in vitro*. The formation of a binary complex between the Rubisco large subunit and chloroplast cpn60 was demonstrated long ago[41,42]. The molar ratio of cpn60 to newly synthesized subunits of Rubisco *in vivo* is also very large[12,13].

The molecular details of what happens to Rubisco while it is associated with the cpn60, and the mechanism by which cpn10 and Mg-ATP facilitate the discharge of the binary complex leading to the formation of active Rubisco, are the least understood aspects of chaperonin function. The hydrolysis of ATP seems to be necessary, because non-hydrolysable analogues of ATP do not support reconstitution. Although the stoichiometry of the reaction is unknown, it is unlikely that the chaperonin system is thermodynamically coupled, with the energy released on hydrolysis of ATP being conserved in the folded native state. Instead, we favour the notion that the hydrolysis of ATP serves some regulatory function and that the energy is ultimately dissipated as heat.

The *E. coli* chaperonins clearly did not evolve to facilitate the folding or assembly, or both, of Rubisco, a protein not normally found in *E. coli*. It follows that the interaction between cpn60 and the partly folded Rubisco cannot be specific for Rubisco alone. Instead, the specificity of the interaction must lie in some so far undefined structural element of the partly folded protein. Because the native protein shows no detectable interaction with cpn60, this structural element must be missing altogether from, or inaccessible in, the native protein. The solution to the problems of substrate specificity and reaction stoichiometry, and to the many other questions raised by this study, await further investigation. □

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1. Ellis, R. J. & Hemmingsen, S. M. *Trends biochem. Sci.* **14,** 339–342 (1989).
2. Mehra, V. *et al. Proc. natn. Acad. Sci. U.S.A.* **83,** 7013–7017 (1986).
3. Vodkin, M. H. & Williams, J. C. *J. Bact.* **170,** 1227–1234 (1988).
4. Torres-Ruiz, J. A. & McFadden, B. A. *Archs. Biochem. Biophys.* **261,** 196–204 (1988).
5. Hemmingsen, S. M. *et al. Nature* **333,** 330–334 (1988).
6. Reading, D. S., Hallberg, R. L. & Myers, A. M. *Nature* **337,** 655–659 (1989).
7. McMullin, T. W. & Hallberg, R. L. *Molec. cell. Biol.* **8,** 371–380 (1988).
8. Picketts, D. J., Mayanil, C. S. K. & Gupta, R. S. *J. biol. Chem.* **264,** 12001–12008 (1989).
9. Sternberg, N. *J. molec. Biol.* **76,** 25–44 (1973).
10. Georgopoulos, C. P. *et al. J. molec. Biol.* **76,** 45–60 (1973).
11. Tilly, K., Murialdo, H. & Georgopoulos, C. *Proc. natn. Acad. Sci. U.S.A.* **78,** 1629–1633 (1981).
12. Ellis, R. J. & van der Vies, S. M. *Photosynthesis Res.* **16,** 101–115 (1988).
13. Roy, H., Cannon, S. & Gilson, M. *Biochim. biophys. Acta* **957,** 323–334 (1988).
14. Gatenby, A. A. *et al. EMBO J.* **7,** 1307–1314 (1988).
15. Bochkareva, E. S., Lissin, N. M. & Girshovich, A. S. *Nature* **336,** 254–257 (1988).
16. Landry, S. J. & Bartlett, S. G. *J. biol. Chem.* **264,** 9090–9093 (1989).
17. Cheng, M. Y. *et al. Nature* **337,** 620–625 (1989).
18. Ostermann, J., Horwich, A. L., Neupert, W. & Hartl, F. U. *Nature* **341,** 125–130 (1989).
19. Lubben, T. H., Donaldson, G. K., Viitanen, P. V. & Gatenby, A. A. *Pl. Cell* **1,** 1223–1230 (1989).
20. Goloubinoff, P., Gatenby, A. A. & Lorimer, G. H. *Nature* **337,** 44–47 (1989).
21. Schneider, G., Lindqvist, Y., Branden, C. I. & Lorimer, G. H. *EMBO J.* **5,** 3409–3415 (1986).
22. Chapman, M. S. *et al. Nature* **329,** 354–356 (1988).
23. Andersson, I. *et al. Nature* **337,** 229–234 (1989).
24. Tabita, F. R. & McFadden, B. A. *J. biol. Chem.* **249,** 3459–3464 (1974).
25. Larimer, F. W. *et al. J. biol. Chem.* **262,** 15327–15329 (1987).
26. Dolgikh, D. A. *et al. FEBS Lett.* **136,** 311–315 (1981).
27. Ohgushi, M. & Wada, A. *FEBS Lett.* **164,** 21–24 (1983).
28. Baum, J. *et al. Biochemistry* **28,** 7–13 (1989).
29. Chory, J. *et al. J. Bact.* **161,** 307–313 (1985).
30. Hendrix, R. W. *J. molec. Biol.* **129,** 375–392 (1979).

31. Pushkin, A. V. *et al. Biochim. biophys. Acta* **704,** 379-384 (1982).
32. Musgrove, J. E. & Ellis, R. J. *Phil. Trans. R. Soc.* B**313,** 419-428 (1986).
33. Pelham, H. R. B. *Cell* **46,** 959-961 (1986).
34. Ellis, R. J. *et al. Biochem. Soc. Symp.* **55,** 145-153 (1989).
35. Fischer, G., Bang, H. & Mech, C. *Biomed. biochim. Acta* **43,** 1101-1111 (1984).
36. Lang, K., Schmid, F. X. & Fischer, G. *Nature* **329,** 268-270 (1987).
37. Freedman, R. B. *Cell* **57,** 1069-1072 (1989).
38. Chandrasekhar, G. N. *et al. J. biol. Chem.* **261,** 12414-12419 (1986).
39. Pierce, J. & Reddy, G. S. *Archs Biochem. Biophys.* **245,** 483-493 (1986).
40. Pierce, J. & Gutteridge, S. *Appl. envir. Microbiol.* **49,** 1094-1100 (1986).
41. Barraclough, R. & Ellis, R. J. *Biochim. biophys. Acta* **608,** 19-31 (1980).
42. Roy, H. *et al. J. cell. Biol.* **94,** 20-27 (1982).

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LETTERS TO NATURE

Lunar basalt breccia identified among Antarctic meteorites

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THE Antarctic meteorite Elephant Moraine (EET) 87521 is a lunar mare basalt breccia. This 30.7-g meteorite was originally identified as a brecciated eucrite[1], but new petrographic evidence from a more representative thin section (Fig. 1) indicates that it is a breccia of mainly very-low-titanium (VLT) basalt, and should be reclassified as a lunar meteorite. The four (or five) Antarctic lunar meteorites collected previously at Allan Hills, Yamato Mountains and MacAlpine Hills (ALHA81005, Y79117/Y793274, Y82192/82193+/−Y86032(?) and MAC88104/88105) have all sampled only the plagioclase-rich lunar highlands[2,3] which dominate the surface of the Moon. EET87521 extends the lunar meteorite collections to include the less abundant basaltic provinces (the maria) of the lunar crust.

Meteorite EET87521 contains lithic and mineral clasts in a matrix of fine-grained breccia and glass. The modal mineralogy, determined from 900 random microprobe analyses of thin section, 87521, 8 (area ~90 mm^2) is: 10% olivine (Fo_{62}–Fo_3; Fo, forsterite); 47% subcalcic augite and augite with Mg/(Mg+Fe) ratios between 0.65 and 0.2 (Fig. 2); 38% calcic plagioclase (An_{68}–An_{95}; An, anorthite)[1]; 4% silica minerals; 1% of ilmenite, chromite, titanium-rich chromite and sulphide. This mineralogy is comparable with many mare basalts[4]. Representative compositions of most minerals are given in Table 1. An estimate of the bulk composition derived from the modal abundance and the mean compositions of the phases present is: 47% SiO_2, <0.8% TiO_2, 11.6% Al_2O_3, <0.4% Cr_2O_3, 19.5% FeO, 0.3% MnO, 8.8% MgO, 10.1% CaO, 0.3% Na_2O, ~100 p.p.m. K_2O (ref. 5). These data are consistent with derivation from a mare basalt source. On the basis of instrumental neutron activation analysis (INAA) data[6], EET87521, 6 seems to contain lunar highlands material, represented by higher normative plagioclase and Mg/(Mg+Fe). The meteorite's oxygen isotope signature[7] is consistent with a lunar origin ($\delta^{18}O = 5.4$, where $\delta^{18}O = ((^{18}O/^{17}O)_{\text{sample}}/(^{18}O/^{17}O)_{\text{standard}}) - 1$; $\delta^{17}O = 2.8$; R. N. Clayton and T. K. Mayeda, personal communication).

The lithic clasts in EET87521, 8 range from a 5-mm, fairly coarse-grained gabbroic fragment with fayalite, iron-rich augite, plagioclase and various oxides (Fig. 1) to small fine-grained graphic intergrowths of augite, fayalite and silica in ilmenite. Magnesian components (Mg/Mg+Fe of 0.65–0.5) are less common (Fig. 2*a*) and occur almost exclusively as mineral clasts. All the plagioclase and pyroxene is shocked. The presence of shock glass indicates pressures in excess of 30 GPa but the plagioclase has generally not been maskelynitized (that is, transformed to a glass by shock pressures in excess of 25 GPa). The textures of the lithic clasts indicate that the sample was shocked to less than the 25–30 GPa necessary[8] for maskelynitization and was invaded by shock glass, perhaps at the time of ejection into an Earth-intercepting trajectory.

The mineralogy of EET87521 differs from the basaltic achondrites with which it was originally identified[1], as it contains much more modal olivine and has abundant subcalcic augite and augite, in addition to pigeonite. The range of mineral compositions is similar to that seen in the polymict achondrites but the Fe/Mn ratios of the mafic minerals (90–100 in olivine and 60–80 in pyroxene) are much higher than those from achondrites (30–53 in olivine and 25–40 in pyroxene[9]). The Fe/Mn

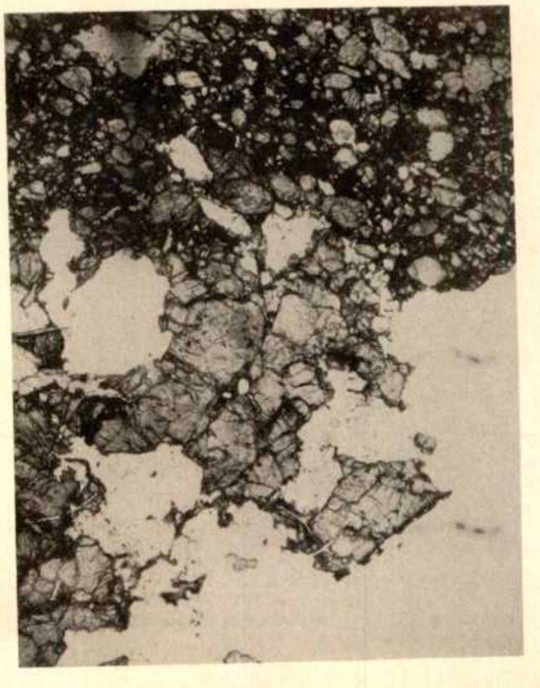

FIG. 1 Augite- and olivine-rich mafic clast and surrounding breccia in meteorite Elephant Moraine 87521, 8, a very-low-titanium basalt breccia. Thin veins of shock glass crosscut both the plagioclase and augite in the lithic clast. Long dimension is ~4 mm, plane-polarized light.

TABLE 1 Representative mineral analyses from EET87521, 8

	Oli1	Oli2	Pyx1	Pyx2	Pyx3	Fel1	Fel2	Fel3	Ilm	Chrm	Ti-sp
SiO	36.6	30.1	52.5	49.6	47.8	45.3	46.3	51.0	0.06	0.02	0.19
TiO_2	0.03	0.11	0.18	0.39	0.76	bd*	0.01	bd	52.3	1.37	27.9
Al_2O_3	0.05	0.14	1.12	1.13	1.23	36.3	34.4	31.6	0.06	18.7	4.2
Cr_2O_3	0.07	bd	0.59	0.37	0.12	na†	na	na	0.34	45.1	2.57
FeO	33.7	64.0	20.2	26.6	29.5	0.42	0.49	0.77	46.1	27.8	63.1
MnO	0.34	0.63	0.32	0.39	0.35	0.03	bd	bd	0.39	0.21	0.3
MgO	30.7	5.9	19.8	11.1	3.6	0.05	0.06	bd	0.5	5.8	0.23
CaO	0.26	0.38	5.2	7.9	14.7	19.0	17.8	14.0	na	na	na
Na_2O	bd	bd	bd	0.06	0.09	0.52	1.02	2.37	na	na	na
K_2O	na	na	na	na	na	0.02	0.03	0.33	na	na	na
Sum	101.76	101.28	99.91	97.59	98.08	101.67	100.11	100.05	99.78	99.1	98.46

Oli, olivine; Pyx, pyroxene; Fel, plagioclase; Ilm, ilmenite; Chrm, chromite; Ti-sp, ulvospinel/magnetite solid solution.
* bd, below detection.
† na, not analysed.

ratios are typical of Apollo and Luna samples and probably of much of the lunar crust. The estimate of the bulk composition resembles the Apollo 17 VLT and Luna 24 VLT basalts[10] but has a low Mg/(Mg+Fe) ratio. The abundant fayalitic olivine in the lithic clasts also shows that this meteorite contains highly fractionated basalts. The incompatible element content (Na and K) seems low in 87521, 8 but rare-earth-element abundances in 87521, 6 (ref. 6) are fairly high and are consistent with a fractionated source.

The number of lunar meteorites from the highlands (four or possibly five) and from the maria (one) matches fairly well with the relative areas of these provinces (83% highlands and 17% maria) on the Moon[11]. The number of samples is small, but this correspondence suggests that impacts on the Moon that are large enough to propel samples to the Earth have been sufficiently frequent to ensure representative random sampling of the lunar crust. The present lunar meteorite suite is a less biased sampling of the entire lunar crust than the combined Apollo and Luna suites, which together enclose <5% of the surface area of the moon[11]. The absence of precise locality information for the lunar meteorites restricts the interpretation of the data, but as samples for testing the generality of models based on the Apollo and Luna suites, they assume enormous importance. Random sampling of the lunar surface by meteorites also implies that two or three of the highlands meteorites are from far-side sites. □

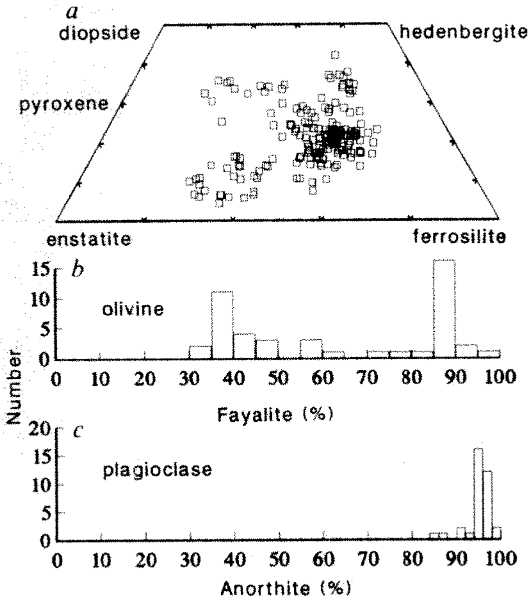

FIG. 2 *a*, Pyroxene compositions from EET87521. Data obtained from random samples of thin section 87521, 8 and analyses of selected mineral grains. *b*, Distribution of olivine compositions in 87521, 8. *c*, Distribution of plagioclase feldspar compositions.

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1. Schwarz, C. & Mason, B. *Antarct. Meteorite Newsl.* **11,** 21 (1988).
2. Score, R. & Mason, B. *Antarct. Meteorite Newsl.* **5** (1982).
3. Score, R. & Mason, B. *Antarct. Meteorite Newsl.* **12** (1989).
4. Meteorites News **4** (1985).
5. Basaltic Volcanism Study Project *Basaltic Volcanism on the Terrestrial Planets* (Pergamon, New York, 1981).
6. Warren, P. H. & Kallemeyn, G. K. *Geochim. cosmochim. Acta* (in the press).
7. Clayton, R. N. & Mayeda, T. K. *Proc. 6th lunar Sci. Conf.* 1761–1769 (1975).
8. Ostertag, R. *Proc. lunar planet. Sci. Conf.* **14,** 364–376 (1983).
9. Desnoyers, C, *Geochim. cosmochim. Acta* **46,** 667–680 (1982).
10. Taylor, G. J. *et al.* in *Mare Crisium: the View from Luna 24* (eds Merrill, R. B. & Papike, J. J.) 303–320 (Pergamon, New York, 1977).
11. Head, J. W. *Rev. Geophys. Space Phys.* **14,** 265–300 (1976).

New double-sheet copper oxide compounds with BiO or TlO bilayers

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ONE of the main families of oxide superconductors comprises compounds with varying numbers of CuO_2 sheets sandwiched between BiO or TlO bilayers[1–5]. The superconducting transition temperature (T_c) in these compounds increases with the number of CuO_2 sheets; for example, T_c is 80–108 K for the double-sheet 2212 phase, and 110–125 K for the triple-sheet 2223 phase. Here we describe a new family of double-sheet copper oxide compounds with BiO or TlO bilayers, which can improve our understanding of the relationship between T_c and the CuO_2 sheets. The new compounds, $Bi_2Sr_2(Ln_{1-x}Ce_x)_2Cu_2O_{10+y}$ and $Tl_2Ba_2(Ln_{1-x}Ce_x)_2Cu_2O_{10+y}$ (where Ln is Sm, Eu or Gd), have double CuO_2 pyramidal sheets between double BiO or TlO layered units, like the 2212 phase, but with a different arrangement of adjacent CuO_2 sheets. The new bismuth compounds (Bi-2222) have a significantly

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lower T_c (~30 K or less) than the 2212 or 2223 phases, even when the charge-carrier (hole) concentrations are comparable. So far, we have not found superconductivity in the Tl-2222 compounds.

We synthesized the materials by solid-state reaction. For the bismuth-based 2222 compounds, $Bi_2Sr_2(Ln_{1-x}Ce_x)_2Cu_2O_{10+y}$, we ground a mixture of stoichiometric amounts of Bi_2O_3, $SrCO_3$, Ln_2O_3 (Ln = Sm, Eu or Gd), CeO_2 and CuO and fired it at 900 °C for 10 h; we then reground it and refired it at 990 °C for 10 h. We pressed the resulting powder into pellets and sintered it at 1,000 °C for 10 h. All firing procedures were done in air and followed by slow cooling. According to the X-ray powder diffraction patterns (see Fig. 1), the materials were nearly single phase for cerium concentrations x in the range 0.03-0.25. We found no evidence in the X-ray diffraction patterns for any superconducting T* phase[6], Bi-2201, 2212 or 2223 phases. These would complicate our interpretation of the observed superconductivity. We prepared the corresponding thallium-based compounds, $Tl_2Ba_2(Eu_{1-x}Ce_x)_2Cu_2O_{10+y}$, by thoroughly mixing stoichiometric quantities of Tl_2O_3, Eu_2O_3, CeO_2 and $BaCuO_2$ with a 10% excess of Tl_2O_3. After grinding, we pressed the mixture into a pellet, wrapped it in gold foil, fired it at 890 °C for 3 h in a sealed quartz tube containing ~1 atm of oxygen, and then slowly cooled it. We readily obtained the desired 2222 phase for compositions near $x = 0.1$, although the resultant material did contain several per cent of the 1212 phase[4,5].

We determined the crystallography and microstructure of both 2222 derivatives using X-ray diffraction and transmission electron microscopy (TEM). Tl-2222 is tetragonal (space group $P4/nmm$) with $a = 3.888(2)$ Å and $c = 17.281(7)$ Å; the unit cell is shown in Fig. 2*a*. Like the 2212 structure (Fig. 2*b*), the 2222 structure contains two pyramidal CuO_2 sheets between the BiO or TlO bilayers but, instead of a layer of Ca^{2+} ions, there is an intervening fluorite-like $(Ln_{1-x}Ce_x)_2O_2$ layer between the two CuO_2 sheets. As a result, the copper in adjacent CuO_2 sheets are displaced by $(a+b)/2$ in the tetragonal cell. Figure 3 is a high-resolution TEM image along the [100] direction of a $Tl_2Ba_2(Eu_{0.9}Ce_{0.1})_2Cu_2O_{10}$ crystal. The simulated-image inset in Fig. 3, which is based on the unit cell derived from X-ray diffraction data, is in good agreement with the actual image. By contrast to the thallium derivative, Bi-2222 is slightly orthorhombic. The unit-cell axes (Table 1) for this phase (compared to Tl-2222) must be rotated ~45° about the c axis and redefined with $a \sim b \sim \sqrt{2}a_p$ (the perovskite unit-cell lattice parameter, a_p is 3.88 Å). Electron diffraction patterns taken on and about the (001) zone axis revealed that there is a strong commensurate superlattice modulation with wavevector $[\frac{2}{9}\ 0\ \frac{1}{2}]$ in the $\sqrt{2}a_p \times \sqrt{2}a_p$ setting. Given the structural similarity of Bi-2222 and Bi-2212, we speculate that the modulation arises from the insertion of one extra oxygen for every nine bismuth atoms in the BiO bilayers. Iodometric titration measurements on Bi-2222 are consistent with this hypothesis (see below). The results of the Rietveld analysis on Bi-2222 samples with stoichiometries $Bi_2Sr_2(Ln_{1-x}Ce_x)_2Cu_2O_{10}$ ($x = 0.15$-0.25) (Ln = Sm or Gd) are listed in Table 1. Unlike 2212 and 2223, neither 2222 derivative contained extensive intergrowths or stacking defects and no twins were observed in cleaved flakes of Bi-2222. We did not perform the neutron diffraction measurement because the compounds contain strongly neutron-absorbing rare-earth elements.

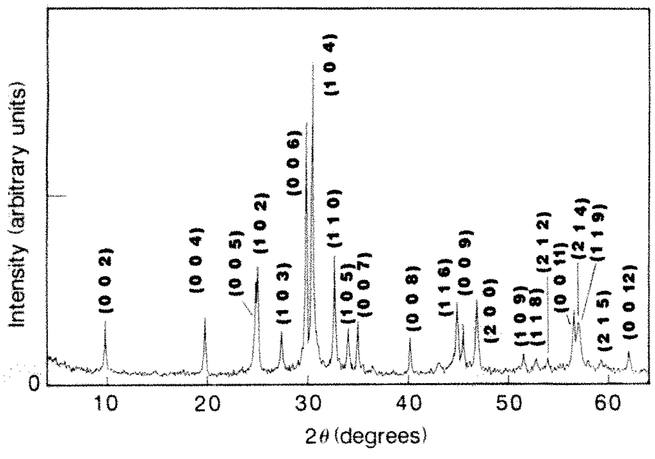

FIG. 1 Powder X-ray diffraction pattern for new Bi-2222 phase.

The 2222 structure is closely related to those of well-known bismuth- and thallium-based compounds[1-5], but has a different stacking of Cu-O sheets. In the T* phase[6] and in the recently discovered Eu-Ba-Ce-Cu-O phase[7], the fluorite-like layers are sandwiched between K_2NiF_4-type units and disordered CuO chains, respectively, rather than between BiO and TlO bilayers as in our 2222 phase. Both of these phases have $T_c \sim 25$-35 K (refs 6, 7). While preparing this work for publication, we became aware of the discovery[8] of a (non-superconducting) thallium-based compound with fluorite-like layers, but this structure has TlO monolayers rather than bilayers.

We probed the superconductivity in the Bi-2222 compounds by measuring the Meissner effect. Figure 4 shows the results measured with 10-Oe field cooling for $Bi_2Sr_2(Ln_{0.85}Ce_{0.15})_2Cu_2O_{10+y}$, (Ln = Sm, Eu and Gd). In all compounds, we observed a substantial Meissner signal with an onset T_c of ~25 K and a magnitude >10% of the ideal value, which ensures the bulk nature of the observed superconductivity. To produce the superconductivity in the Bi-2222 samples, extensive oxygen annealing was necessary. We oxygenated the samples for which the data are plotted in Fig. 4 under 80 atm of oxygen at 550-600 °C for 10 h. By iodometric titration, we determined the amount of extra oxygen y, which is postulated to reside in the BiO bilayer, to be $y \sim 0.24 \pm 0.02$. For the same oxygenation conditions, the y value was approximately constant for samples with $x = 0.15$-0.25, but decreased for compounds with less cerium. Because the valence of the cerium ion in the fluorite-like layer is very likely[9] to be 4+, the effective $[CuO]^{p+}$ charge or the concentration p of chemically doped holes per

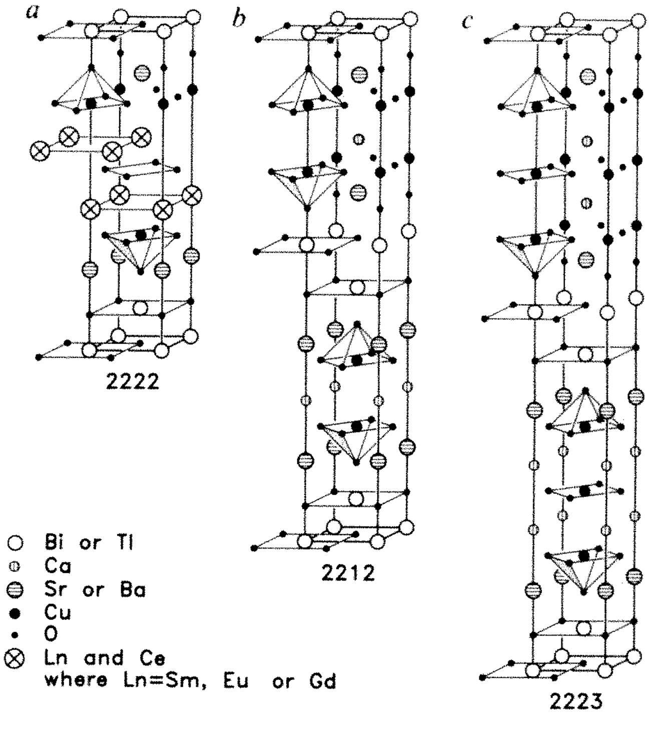

FIG. 2 Nominal unit cells for the *a*, 2222; *b*, 2212; and *c*, 2223 phases.

TABLE 1 Atomic coordinates of the new Bi-2222 compound

		$Bi_2Sr_2(Sm_{1-x}Ce_x)_2Cu_2O_{10+y}$			$Bi_2Sr_2(Gd_{1-x}Ce_x)_2Cu_2O_{10+y}$
		$x=0.25$	$x=0.20$	$x=0.15$	$x=0.15$
a		5.4630 (10)	5.4670 (22)	5.4636 (13)	5.4445 (9)
b		5.4730 (10)	5.4708 (22)	5.4723 (13)	5.4573 (9)
c		17.887 (2)	17.919 (2)	17.921 (2)	17.913 (2)
a/*b*		0.998	0.999	0.998	0.997
Bi	in $(0\,\frac{1}{4}\,z)$	$z=0.5854$ (4)	0.5853 (7)	0.5842 (6)	0.5854 (7)
Sr	in $(0\,\frac{1}{4}\,z)$	0.2671 (9)	0.266 (1)	0.266 (1)	0.266 (1)
Cu	in $(0\,\frac{1}{4}\,z)$	0.833 (1)	0.823 (2)	0.837 (3)	0.836 (2)
Sm, Ce	in $(0\,\frac{1}{4}\,z)$	0.0687 (6)	0.0693 (8)	0.0679 (8)	0.0689 (8)
O(1)	in $(0\,\frac{1}{4}\,z)$	0.399 (5)	0.419 (7)	0.403 (5)	0.410 (6)
O(2)	in $(0\,\frac{1}{4}\,z)$	0.710 (5)	0.715 (6)	0.718 (9)	0.712 (10)
O(3)	in $(\frac{1}{4}\,0\,z)$	0.841 (3)	0.835 (4)	0.841 (3)	0.841 (3)
O(4)	in $(\frac{1}{4}\,0\,0)$				
B_{metal}	(Å^2)	2.3 (2)	1.9 (2)	2.0 (2)	2.1 (2)
B_O	(Å^2)	4.9 (13)	2.4 (13)	0.2 (12)	2.3 (14)
R_{wp}	(%)	11.70	12.58	11.79	11.86
R_I	(%)	6.58	8.57	6.66	7.80

Space group *Cmma* with $\sqrt{2}a_p \times \sqrt{2}a_p \times c$ unit cell. The values in parentheses represent the uncertainty in the last digit(s). B_{metal} and B_O, are the Debye–Waller factors for metal and oxygen, respectively. R_{wp} and R_I are the *R*-factors for the weighted sum and intensities, respectively.

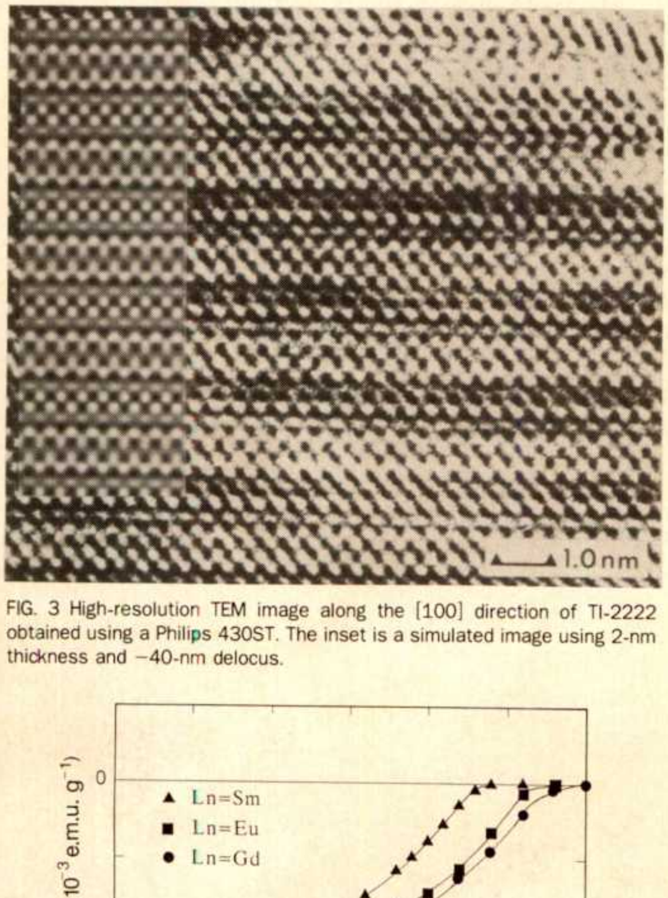

FIG. 3 High-resolution TEM image along the [100] direction of Tl-2222 obtained using a Philips 430ST. The inset is a simulated image using 2-nm thickness and −40-nm delocus.

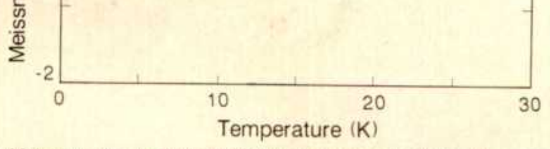

FIG. 4 Meissner signals of Bi-2222 phase compounds, $Bi_2Sr_2(Ln_{0.85}Ce_{0.15})_2Cu_2O_{10.2}$ (Ln=Sm, Eu and Gd). We plot the ratio of magnetization *M* to applied field *H*, for $H=10$ Oe. The Curie-like contribution of magnetic lanthanide ions was subtracted.

copper atom is given by the relation, $p=y-x$. The Bi-2222 samples all have positive *p* values in the range 0–0.1, indicating that the charge carriers are holes and not electrons, by contrast to cerium-doped Ln_2CuO_4 compounds[9,10]. This is also consistent with preliminary results of thermopower measurements, which show the existence of positive charge carriers. The cerium ions stabilize the fluorite-like Ln_2O_2 layer, as observed in other families of layered copper oxides[6,9], and also reduce the total number of holes introduced by the excess oxygen. In fact, highly cerium-doped compounds ($x=0.25$) showed semiconducting or insulating behaviour in the temperature dependence of their resistivity.

According to iodometric-titration analysis, the above mentioned oxygenation procedure can increase the hole concentration up to $p=0.10$ in Bi-2222, nevertheless T_c remains around 25 K for Ln = Sm, Eu or Gd. It is more difficult to characterize the stoichiometry or control the oxygen content of Tl-2222 than it is for Bi-2222, but according to our preliminary data the samples show metallic transport behaviour.

The low T_c observed in Bi-2222 is in sharp contrast to the higher T_cs observed in the 2212 compounds (80–108 K) and the 2223 compounds (110–125 K) with comparable values of *p* (≈ 0.1–0.2). We note that the 2222 and 2223 compounds have a repeat unit of nearly the same size between the BiO or TlO bilayers: the fluorite-like layer in the 2222 phase contains one O_2 sheet sandwiched by two lanthanide layers, which corresponds well to the CuO_2 sheet between the calcium layers in the 2223 phase (Fig. 2*a*, *c*). According to the empirical law for charge-carrier types[9,10], an isolated CuO_2 sheet with no apical oxygen cannot sustain holes as charge carriers but can be doped with electrons, whereas the reverse is true for the pyramidal CuO_2 sheet. Therefore, the hole-type carrier motion is probably primarily restricted to the pyramidal CuO_2 sheets in 2223. The distance $d=5.9$ Å between the hole-conducting pyramidal CuO_2 sheets in 2222 ($T_c=25$ K) is comparable to that ($d=6.4$ Å) in 2223 ($T_c=110$–125 K). Why then is T_c so low in the 2222 compound? One of the most significant differences between the 2222 compound and the 2212 or 2223 compounds may be the relative arrangement of the adjacent pyramidal sheets (Fig. 2). Another factor may be the larger in-plane Cu–O bond lengths[11] (induced by the presence of the fluorite-like layers) in the 2222 compounds, which might decrease T_c. Instead of depending simply on the number of Cu–O sheets between the BiO and TlO bilayers, it seems that T_c depends strongly on a combination of factors, including the stacking pattern of these sheets, the Cu–O bond lengths and the carrier concentration. □

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1. Maeda, H., Tanaka, Y., Fujutomi, M. & Asano, T. *Jap. J. appl. Phys.* **27** L209–211 (1988).
2. Sheng, Z. Z. & Hermann, A. M. *Nature* **332,** 138–139 (1988).
3. Torrance, J. B. *et al. Solid St. Commun.* **66,** 703–706 (1988).
4. Beyers, R. *et al. Appl. Phys. Lett.* **53,** 432–435 (1988).
5. Ihara, H. *et al. Nature* **332,** 623–624 (1989).
6. Sawa, H. *et al. Nature* **337,** 347–348 (1989).
7. Sawa, H. *et al. J. phys. Soc. Japan* **58,** 2252–2255 (1989).
8. Martin, C. *et al. Mod. Phys. Lett.* (in the press).
9. Tokura, Y. *et al. Phys. Rev.* B**39,** 9704–9707 (1989).
10. Tokura, Y., Takagi, H. & Uchida, S. *Nature* **337,** 345–347 (1989).
11. Whangbo, M.-H., Kang, D. B. & Toradi, C. C. *Physica* C**158,** 371–376 (1989).

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Wave damping by rain

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It is common knowledge among seafarers that rain "knocks down the sea"[1,2] or that surface-wave breaking is suppressed by heavy rain. Although no quantitative data exist, several mechanisms have been proposed that might account for the attenuation of waves by rain. The most likely seems to be that raindrops produce turbulence in the water which then helps to dissipate the waves. Here we describe laboratory experiments which show that, in the absence of wind, rain preferentially damps short-wavelength gravity waves. The observed damping rates are, however, lower than earlier predictions[3,4]. The suppression of short waves has an effect on the sea surface similar to that of an oil film, leading to a reduction in the breaking of long waves. The phenomenon is important because it helps to provide insight into other aspects of wave breaking or wave suppression, and because of its bearing on the detection and estimation of rainfall at sea from ambient noise levels[5], and of the measurement of winds using radar, as scatterometer estimates of wind speed are known to be biased when it rains[6]. Wind speed is determined from an empirical relation with the observed strength of the backscattered radar signal. Although the signal may be influenced by atmospheric effects in rain, the very-small-scale roughness of the surface caused by rain drops or, as we shall show, the reduction of the 10-cm-scale waves, may also affect the signal and hence the wind-speed estimate.

Observations[7] and analytical studies[8] have shown that, in windy conditions, rain may enhance the net stress of the wind on the sea surface and, at first sight, suppression of wave breaking is therefore unexpected. The phenomenon is, however, far from simple. Rain modifies the structure of the atmospheric boundary layer[9]. The differential velocity of raindrops striking the moving sea surface induces both normal and tangential stresses on the water[3]. The net stress contributes both to waves and mean currents. The impact of raindrops produces capillary gravity ripples[10], sub-surface vortex rings[2] and vertical mixing[3,4], bubbles[11,12] and the generation of secondary drops—for example, when large bubbles burst on returning to the surface. A thin fresh-water mixing layer may build up at the sea surface[13], with density gradients restricting diffusion[14].

The full problem is very complex and we therefore conducted laboratory experiments in which only the most essential elements are retained—the rain (all of the same drop size) and the waves on fresh water—neglecting, for the present, the added complexity of wind and salinity. We used a wave tank that is 14 m long, 0.6 m wide and 0.6 m deep (Fig. 1). We produced drops of radius $R = 1.6$ mm with an array of 3,200 hyperdermic needles mounted 25 mm apart, in a triangular pattern and set in the base of waterfilled trays mounted 1.25 m above the water surface. The 'artificial rain' covered a 2-m-long section spanning the wave tank. Drops reached the water surface with a fall speed W of 4.5 m s^{-1}, about half their terminal fall speed[15], but with an intensity I of 600 mm hr^{-1}, greater than usual under natural conditions. The net flux of kinetic energy per unit area of the water from the artificial rain, $\frac{1}{2}\rho W^2 I$, where ρ is the density of water in raindrops, was comparable to that of natural rain. We produced waves of frequency $\sigma = 1.8$–4.2 Hz using a wedge at one end of the tank. These waves passed through a capacitance wire gauge, through the 'rain' section and then through a second gauge before finally being absorbed on a 'beach'. In our first, 'no-rain', experiments we used the gauge outputs averaged over 40 waves to determine a damping coefficient δ (a function of σ, or of wavenumber k) from $a_2 = a_1 \exp(-\delta d)$, where a_1, a_2 are the wave amplitudes at the first and second gauges and d is their separation. Damping is the result of viscous dissipation at the sides and bottom of the tank[16], and in the interior and at the surface of the fluid[17]. We found the second source of damping to be dominant, with a value larger than that of molecular viscosity at a clean water surface

$$\delta = 4\sigma^5 \nu / g^5 \tag{1}$$

(where g is the acceleration due to gravity), but well below that of a surface at which tangential motion is entirely suppressed (Fig. 2). (As the water surface was not 'pre-cleaned' we expected some slight contamination.) At frequencies ≤ 16 rad s^{-1} (2.5 Hz), measurements were affected by wave reflection from the beach and the presence of cross-waves. Measurements of short waves (frequencies >25 rad s^{-1} or 4 Hz) were affected by errors due to capillary effects at the wave gauges.

Our experiments with rain showed enhanced damping. To derive the attenuation rate Δ for surface waves in the rain (Fig. 3*a*), we used the attenuation rate δ to account for wave damping in the area between the gauges but outside the 2-m rain zone, and also corrected for side and bottom damping in the rain zone. We have estimated the damping owing to (1) the pressure of the rain on the advancing waves, (2) the tangential stress on the moving water surface that is due to the relative speeds of rain drops entering the water, and (3) the momentum loss of the waves through the interaction with capillary gravity waves produced by raindrops is not appreciable. This net contribution,

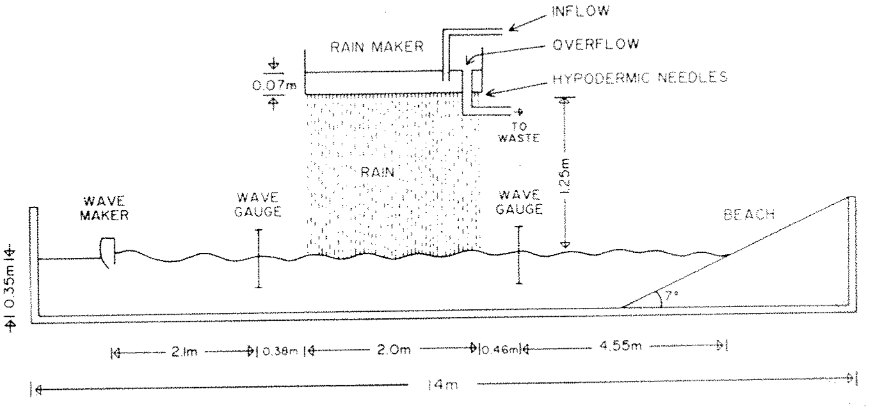

FIG. 1 The experimental apparatus. Artificial rain is produced from an array of hypodermic needles set in the bottom of a tray and falls onto waves produced by a wavemaker. The wave gauges are 0.315-mm-diameter capacitance wire probes located on the centre line of the tank.

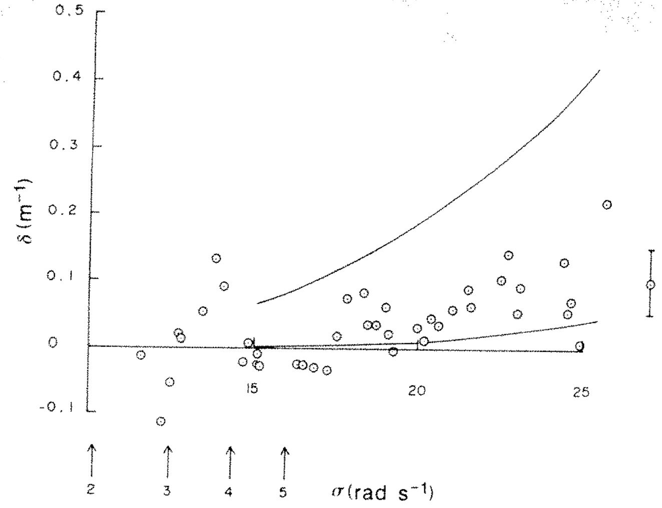

FIG. 2 The damping rate δ as a function of frequency σ of waves in the absence of rain. We have corrected the data for the effects of side walls and tank bottom. The vertical bar indicates the possible uncertainty associated with each estimate. The arrows marked 2–5 indicate the frequencies at which cross-waves may occur; the number is the mode number. The lower curve is the predicted curve with a free upper surface and $\nu = 1.1\times10^{-6}\ \mathrm{m^2\ s^{-1}}$. The upper curve is that predicted for a contaminated water surface with no tangential motion possible.

assuming the molecular viscosity and no turbulent motion, is $\leq$10% of the observed damping. By analogy with equation (1) the damping by vertical mixing may be represented

$$\Delta = 4\sigma^5 \nu_e / g^3 \qquad (2)$$

where ν_e is an effective viscosity. Figure 3*b* shows values of ν_e derived from equation (2), which have a mean value of $2.84\times10^{-5}\ \mathrm{m^2\ s^{-1}}$ for σ in the range 16.0–25.2 rad s⁻¹. The curve in Fig. 3*a* shows Δ as a function of σ for this mean value. We found no significant dependence of ν_e on wave steepness ($0.05 < a_1 k < 0.3$).

Yakimov[10] has suggested $\nu_e = bLI[1-\exp(-2\sigma^2 L/g)]$, where b is estimated to be ~15 and represents the mass carried down in the water by each raindrop divided by the mass of the drop, and L is the depth to which mixing extends, assumed[10] to be ~5 cm. Agreement with the observed ν_e requires $L = 1.2$ cm, which is less than the observed mixing-layer thickness; the formula seems to overestimate ν_e. ($L = \nu_e/I$ corresponds to the boundary-layer thickness predicted for flow at rate I through a porous wall into a fluid of viscosity ν_e. Using our experimental parameters, $L = 17$ cm, which is not inconsistent with depths observed when artificial rain was dyed.) Manton's[3] proposal, $\nu_e = RI^{1/6}W^{5/6}$, gives ν_e in terms of known parameters but it overestimates $\nu_e(1.31\times10^{-3}\ \mathrm{m^2\ s^{-1}})$.

We can judge the possible importance of rain damping in modifying the wave field by comparing the observed Δ with the growth rate of waves that is due to wind[18], $\beta = 0.04k\ (u_*/c)^2$, where u_* is the friction velocity, which is ~0.04 times the wind speed 10 m above the water surface, W_{10}, and $c = \sigma/k$ is the phase speed. Assuming the gravity-wave dispersion relation, $\sigma^2 = gk$, β and Δ (given by equation (2)) are equal when $\sigma\nu_e = 1.6\times10^{-5}W_{10}^2$. In a wind of 6 m s⁻¹, damping at the rate observed in the experiments would exceed the growth induced by the wind for waves of frequency >20 rad s⁻¹ (wavelength $\leq$0.16 m).

A further contribution to short-wave dissipation in windy conditions is due to the enhancement of wind stress by rain[7,8] and a consequently increased surface wind drift current. The rain-induced stress $\tau_r \simeq 0.85\rho I W_{10}$ (ref. 8), together with the wind stress τ lead to a surface drift q proportional to $[(\tau+\tau_r)/\rho]^{1/2}$, which increases with rain intensity. This then leads to a reduction in the amplitude, $a = (c^2/2g)(1-q/c)^2$, at which short gravity waves break[19]. This small-scale breaking represents an increased rate of energy loss from the short waves, and to waves of smaller mean amplitude.

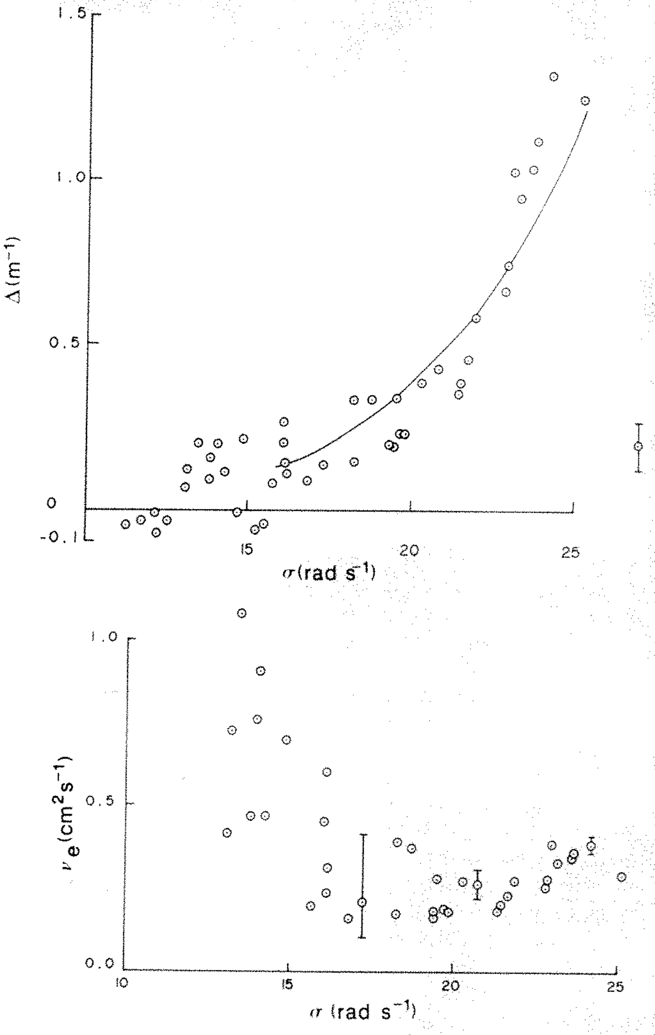

FIG. 3 *a*, The damping rate Δ as a function of frequency σ of waves in rain, with side-wall and tank-bottom effects removed. The vertical bar indicates the possible uncertainty associated with each estimate, and the curve is that given by equation (2) with the average value of ν_e. *b*, The effective viscosity ν_e as a function of wave frequency σ, using only values of $\Delta > 0$ in *a*. The vertical bars indicate the possible uncertainty associated with each estimate. Points for $\sigma < 16$ rad s⁻¹ are seriously affected by cross-waves and edge-waves at the beach and should be discounted. There seems to be no significant trend in ν_e for σ in the range 16–25.2 rad s⁻¹.

Short waves with $k > g/(25u_*^2)$, which include those severely damped by rain, constitute a major component of the roughness of the surface, which is important in the transfer of momentum from the wind to the larger waves[20]. Although raindrops, and splashes, roughen the sea surface at the scales of capillary gravity waves, the attenuation of the short gravity waves caused either by the enhanced turbulence observed in experiments or by their dissipation through small-scale breaking as indicated above, will reduce the surface roughness and hence the momentum transfer to the longer waves, and so diminish the frequency of the more violent form of breaking associated with the longer waves, which are a hazard to mariners. By reducing the short waves, rain calms the sea in a way similar to oil[21]. In the absence of wind, as in the experiments, heavy rain tends rapidly to attenuate short waves typical of lakes or fjords, and contributes to the glassy appearance of their surfaces commonly observed once the rain has stopped. Further experiments to determine the functional relation of I and W are necessary, as is a comprehensive study of rain and wave interactions in windy conditions[6]. □

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1. Coles, A. K. *Heavy Weather Sailing* (Granada, London, 1967).
2. Reynolds, O. *Papers on Mechanical and Physical Subjects* Vol. 1, 86 (Cambridge Univ. Press, 1900).
3. Manton, M. J. *Geophys. Fluid Dyn.* **5**, 249–260 (1973).
4. Yakimov, Yu. L. *Izv. Sibirskogo Otdeleniya Akad. Nauk. CCCP* **5**, 125–126 (1959).
5. Scrimger, J. A., Evans, D. J. & Yee, W. *J. acoust. Soc. Am.* **85**, 726–731 (1989).
6. Black, P. G., Gentry, R. C., Cardone, V. J. & Hawkins, J. D. *Adv. Geophys.* **27**, 198–277 (1985).
7. Van Dorn, W. *J. Mar. Res.* **12**, 249–276 (1953).
8. Caldwell, D. R. & Elliott, W. P. *J. phys. Oceanogr.* **1**, 145–148 (1971).
9. Caldwell, D. R. & Elliott, W. P. *Boundary Layer Met.* **3**, 146–149 (1972).
10. Houk, D. F. & Green, T. *J. geophys. Res.* **81**, 4482–4484 (1976).
11. Thorpe, S. A. & Hall, A. J. *Continental Shelf Res.* **1**, 353–384 (1983).
12. Pumphrey, H. C. & Crum, L. A. in *Sea Surface Sound* (ed. Kerman, B. R.) (Klumar, Dordrecht, 1987).
13. Katsaros, K. & Buettner, K. J. K. *J. appl. Met.* **8**, 15–18 (1969).
14. Green, T. & Houk, D. F. *J. Fluid Mech.* **90**, 569–588 (1979).
15. Laws, J. O. *Trans. Am. geophys. Un.* **22**, 709–721 (1941).
16. Hunt, J. N. *La Houille Blanche* **7**, 836–841 (1952).
17. Dorrestein, R. *Proc. Kon. Acad. Wetensch. Ser.* B**54**, 260–272 (1951).
18. Plant, W. J. *J. geophys. Res.* **87**, 1961–1967 (1982).
19. Phillips, O. M. & Banner, M. L. *J. Fluid Mech.* **66**, 625–640 (1974).
20. Phillips, O. M. *The Dynamics of the Upper Ocean* 2nd edn (Cambridge Univ. Press, 1977).
21. Scott, J. C. in *Oceanic Whitecaps* (eds Monahan, E. C. & MacNiocaill, G.) 159–165 (Reidel, Dordrecht, 1986).

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Nitrification rates and ^{15}N abundances of N_2O and NO_3^- in the western North Pacific

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NITROUS oxide plays an important part in atmospheric chemistry; it is a 'greenhouse' gas and has the potential to destroy ozone. Concentrations of N_2O in surface waters of the oceans are generally close to equilibrium with the atmosphere, but sub-surface sea water is supersaturated with N_2O[1–5]. The oceans are now regarded as a source[4] for atmospheric N_2O by production through the oxidation of ammonium ('nitrification')[1,3], except in extremely oxygen-deficient waters[6]. Nevertheless, the possibility that N_2O might be produced through the reduction of nitrate ('denitrification') cannot be ruled out[5,7]. The distribution of ^{15}N in atmospheric and oceanic N_2O can be used to investigate the behaviour of N_2O in the oceans[8,9]. Here we report simultaneous measurements of the nitrification rates and nitrogen isotope data for N_2O and NO_3^- produced in the western North Pacific Ocean. The amount of N_2O produced by nitrification is much lower than that expected from the production of NO_3^-. The N_2O in the oxygen-deficient layer is more enriched in ^{15}N than NO_3^-. These results imply that the contribution of nitrification to the production of N_2O is lower than previously thought and that denitrification is primarily responsible for the production of N_2O.

During a cruise (August–September 1983) of the RV *Hakuho Maru* in the western North Pacific Ocean we collected seawater samples at depths ranging from the surface to 4,800 m at Station C (45° 09′ N, 160° 18′ E) and from the surface to 4,000 m at Station E (28° 06′ N, 152° 56′ E). Figures 1 and 2 show the vertical profiles of the N_2O concentration, $\delta^{15}N$ values and the potential production rate of N_2O and NO_3^- by nitrification and the dissolved oxygen at Stations C and E, respectively. The dissolved N_2O concentrations in surface water (9.0 nM at Station C and 5.6 nM at Station E) agree well with the equilibrium values estimated using solubility data of N_2O (ref. 13). The maximum concentrations of N_2O occur at a depth of 700 m at Station C and at 1,300 m at Station E, where the dissolved oxygen is a minimum. There is a strong correlation between the N_2O and NO_3^- concentrations and a negative correlation between the N_2O and O_2 concentrations at both stations, as shown in Fig. 3. These correlations are similar to those reported for other areas of the oceans[1–5,7] except for the deep-water column at Station E where a water mass enriched in NO_3^- and depleted in N_2O (ref. 4) is mixed in. The lines in Fig. 3 'turn over' because of the depth dependence—N_2O is generally depleted in bottom waters.

The $\delta^{15}N$ value of the dissolved N_2O in surface water is

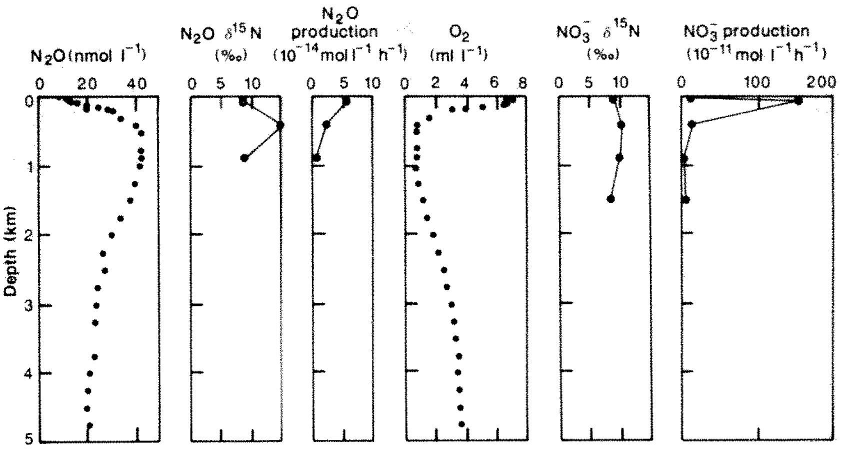

FIG. 1 The vertical profiles at Station C of (from left to right) N_2O, $\delta^{15}N$ of N_2O, the potential production rate of N_2O by nitrification, dissolved O_2, $\delta^{15}N$ of NO_3^- and the potential production rate of NO_3^- by nitrification.

METHODS. We determined the N_2O concentration of the water samples, which had been stored in 100-ml air-tight glass containers, using the method given in ref. 10. We determined using a method we have described elsewhere[9], the ^{15}N content ($\delta^{15}N = ((^{15}N/^{14}N)_{sample}/(^{15}N/^{14}N)_{standard}) - 1$, the standard is N_2 purified from the atmosphere) of N_2O in 100-l samples collected at several depths at the two stations; to determine the $\delta^{15}N$ of NO_3^- we used the method in ref. 11 (5-l samples). We estimated (on board) the production rates of N_2O and NO_3^- by nitrification using ^{15}N-tagged NH_4^+. We added 5 μmol of NH_4^+ (99.8 atom% ^{15}N) to a 5-l water sample contained in an air-tight vessel. After incubating the sample at approximately the *in situ* temperature for 48 h (in the dark), we added 5 ml of 0.1M $HgCl_2$ to stop the bacterial reactions. We used the same method to remove the N_2O as we used to remove N_2O having a natural abundance of ^{15}N. We determined the $\delta^{15}N$ of the 50–200 nmol N_2O so collected by adding a known amount (~1 μmol) of N_2O having $\delta^{15}N = -2.9$‰. We measured the $\delta^{15}N$ of NO_3^- using the same method as we used for NO_3^- having a natural ^{15}N abundance. At the start of the incubation the NH_4^+ is ~1 μM, about ten times larger than the saturation concentration for nitrification[12]. The rates that we calculate correspond to the potential production rates of N_2O and NO_3^-. The precision of the measurements are ±2%, ±0.2‰ and ±3% (1σ) for N_2O, $\delta^{15}N$ and production rates, respectively.

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concentration would be proportional to the apparent oxygen utilization.

The nitrous oxide at the concentration maxima of both stations is found to be more enriched in ^{15}N than that in equilibrium with atmospheric N_2O and NO_3^-. The enrichment in ^{15}N of N_2O that we found has been observed in the oxygen-minimum layer of the eastern tropical Pacific Ocean, where denitrification prevails[9]. The δ^{15}N values of N_2O correlate with those of NO_3^- (Fig. 4). This indicates that most of the N_2O is involved in the NO_3^- reduction system. Nitrous oxide is consumed as well as produced in the NO_3^- reduction system. As a result, the δ^{15}N of N_2O is determined by the fractionation factors during both production and consumption. We have observed a kinetic nitrogen isotope fractionation factor during the reduction of N_2O by denitrifying bacteria of up to 1.028 in terms of the rate-constant ratio of the isotope species, $k(^{14}\text{N})/k(^{15}\text{N})$ (ref. 24). If the ^{15}N enrichment of substrates is greater during consumption than during production, the enrichment in ^{15}N of N_2O relative to NO_3^- should result. The concentration and the δ^{15}N value of N_2O are consequently determined by those of NO_3^-. □

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1. Yoshinari, T. *Mar. Chem.* **4,** 189–202 (1976).
2. Elkins, J. W., Wofsy, S. C., McElroy, M. B., Kolb, C. E. & Kaplan, W. A. *Nature* **275,** 602–606 (1978).
3. Cohen, Y. & Gordon, L. I. *Deep Sea Res.* **25,** 509–524 (1978).
4. Cohen, Y. & Gordon, L. I. *J. geophys. Res.* **84,** 347–353 (1979).
5. Pierotti, D. & Rasmussen, R. A. *Tellus* **32,** 56–72 (1980).
6. McElroy, M. B., Elkins, J. W., Wofsy, S. C. & Yung, Y. L. *Rev. Geophys. Space Phys.* **14,** 143–150 (1976).
7. Hahn, J. *Denitrification, Nitrification and Atmospheric Nitrous Oxide* (ed. Delwiche, C. C.) 191–240 (Wiley-Interscience, New York, 1981).
8. Yoshida, N. & Matsuo, S. *Geochem. J.* **17,** 231–239 (1983).
9. Yoshida, N., Hattori, A., Saino, T., Matsuo, S. & Wada, E. *Nature* **307**, 442–444 (1984).
10. Cohen, Y. *Analyt. Chem.* **49,** 1238–1240 (1977).
11. Liu, K. K. thesis, Univ. California (1979).
12. Hashimoto, L. K., Kaplan, W. A., Wofsy, S. C. & McElroy, M. B. *Deep Sea Res.* **30,** 575–590 (1983).
13. Weiss, R. F. & Price, B. A. *Mar. Chem.* **8,** 347–359 (1980).
14. Yoshida, N. *Nature* **335**, 528–529 (1988).
15. Wada, E., Koike, I. & Hattori, A. *Mar. Biol.* **29,** 119–124 (1973).
16. Miyazaki, T. E., Wada, E. & Hattori, A. *Deep Sea Res.* **20,** 571–577 (1973).
17. Miyazaki, T. E., Wada, E. & Hattori, A. *Mar. Sci. Commun.* **1,** 381–394 (1975).
18. Hattori, A., Goering, J. J. & Boisseau, D. B. *Mar. Sci. Commun.* **4,** 138–151 (1978).
19. Olson, R. J. *J. Mar. Res.* **39,** 203–226; **39,** 227–238 (1981).
20. Ward, B. B. & Zafiriou, O. C. *Deep Sea Res.* **35,** 1127–1142 (1988).
21. Goreau, T. J. *et al. Appl. envir. Microbiol.* **40,** 526–532 (1980).
22. Lipschultz, F. *et al. Nature* **294,** 641–643 (1981).
23. Alldredge, A. L. & Cohen, Y. *Science* **235,** 689–691 (1987).
24. Yoshida, N. thesis, Tokyo Inst. Technology (1984).
25. Strickland, J. D. H. & Parsons, T. R. *A Practical Handbook of Seawater Analysis, Bull Fisheries Res. Board Can.* No. 167 (1972).

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Evidence for a change in the periodicity of tropical climate cycles at 2.4 Myr from whole-core magnetic susceptibility measurements

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THE Earth's orbit about the Sun is influenced by gravitational interactions with the Moon and other planets. The resulting orbital perturbations give rise to cyclical variations in orbital eccentricity, obliquity and precession with periods of 100, 41, 19 and 23 kyr, respectively[1]. These variations are climatically important because they affect the seasonal and latitudinal distribution of solar radiation. Magnetic-susceptibility measurements in deep-sea sediment cores can be used as a sensitive indicator of temporal variations in the concentration of terrigenous material supplied to the sea bed. Here we use this approach to study the evolution of cycles of terrigenous sedimentation at Ocean Drilling Program sites in the Arabian Sea and the eastern tropical Atlantic. We show, from spectral analyses of the two susceptibility time series spanning the past 3.2–3.5 Myr, that the records are driven strongly by orbital forcing. Before 2.4 Myr before present (BP), both records are strongly influenced by the 23- and 19-kyr periodicities; but after 2.4 Myr, they are both significantly influenced by the 41-kyr periodicity. This shift coincides with the beginning of major Northern Hemisphere glaciation, which indicates, for the Indian Ocean site, that the supply of terrigenous sediment (carried by monsoonal winds) has responded to the rapid increase in ice cover at this time. We cannot, however, exclude possible effects of changes in bottom-water circulation on the susceptibility record of the Atlantic site.

Statistical analyses of the foraminiferal δ^{18}O record from deep-sea cores (largely a measure of glacial ice volume[2]) demonstrate that the growth and decay of ice sheets have been strongly modulated by orbital insolation variations[3]. Similarly, other major components of climate[4–8] vary predominantly at orbital periodicities. However, measurement of conventional palaeoclimatic indicators (for example, oxygen and carbon isotopes, carbonate, opal and terrigenous fraction content) in deep-sea sediments is time consuming; consequently, palaeoclimate indicators that can be measured more easily are needed.

Magnetic susceptibility is the ratio of induced magnetization to an applied weak magnetic field and is proportional to magnetic mineral concentration, which is typically a trace component of the terrigenous fraction[9–11]. Susceptibility measurements are extremely rapid, and whole cores can be measured continuously using a pass-through loop sensor. Provided that susceptibility is correlated to terrigenous-fraction variations, these measurements can offer a highly efficient means of acquiring high-resolution palaeoclimate data.

The major aims of Ocean Drilling Program Legs 108 (eastern tropical Atlantic) and 117 (Arabian Sea) were to study the late Neogene evolution of northwest African climates and the Asian

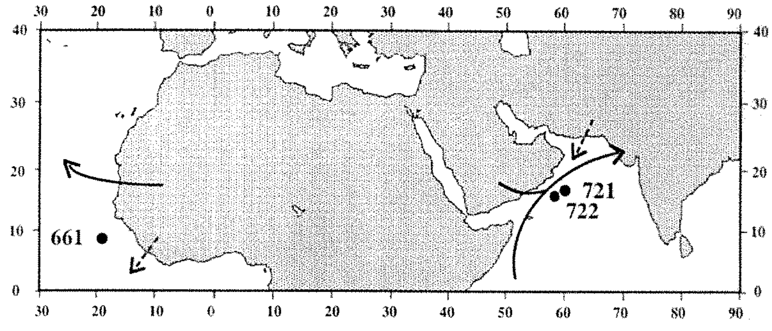

FIG. 1 Locations of ODP Leg 108 Site 661 and ODP Leg 117 Site 721. Solid arrows depict the modern summer monsoonal dust trajectories, dashed arrows depict the winter trajectories[13,17].

monsoon. In the case of the Asian monsoon, summer heating result in the development of an intense low-pressure cell over the Tibetan Plateau; consequently, in the northwest Arabian Sea, strong moisture-laden southwesterly winds transport abundant aeolian detritus[12] to the Arabian Sea region and bring monsoon rains to southern Asia[13]. The northwest African monsoon is driven by the same mechanism: summer heating of North Africa drives the cyclonic inflow of moisture-laden air into the Sahel and south Sahara.

Both general circulation model (GCM) experiments[14] and analyses of aeolian and biogenic components in deep-sea cores[5,6,14] have demonstrated that seasonal insolation variations resulting from orbital precession have modulated the intensity of the Asian and African monsoons. Precession affects low-latitude summer insolation at periodicities of 23 and 19 kyr (ref. 1); maximum summer insolation is achieved when the summer solstice coincides with perihelion, and palaeoclimate data have demonstrated that the African and Asian monsoon systems were intensified at these times[5,6].

Site 721 (Fig. 1) is located in the Arabian Sea in water of depth 2,028 m near the crest of the Owen Ridge, which rises 2,000 m above the surrounding bathymetry. Satellite imagery[15] and sediment-core studies[15,16] demonstrate that terrigenous sedimentation in the Arabian Sea is dominantly aeolian. Site 661 (Fig. 1) is located in 4,013-m water depth on the eastern slope of the Kane Gap in the eastern tropical Atlantic. Today the area receives a substantial supply of aeolian dust from the African winter dust plume[17]. The fact that it is not situated on a bathymetric high suggests that carbonate dissolution and advective sediment supply may have been significant sedimentary processes.

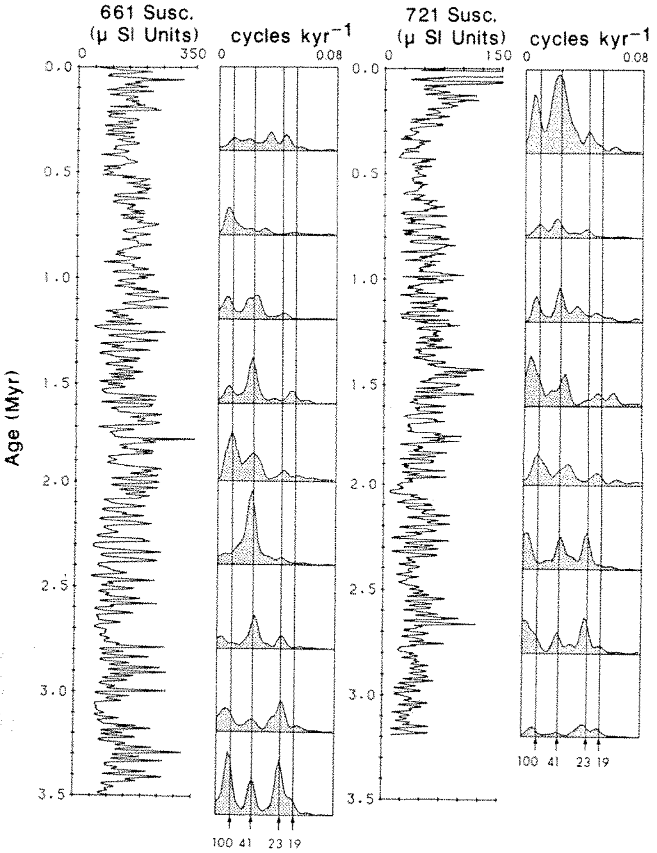

FIG. 2 Susceptibility (Susc.) time series for Site 661 (eastern equatorial Atlantic) and Site 721 (Arabian Sea). The time series were divided into 0.4-Myr intervals and spectral analysis (Sande-Tukey radix-two fast fourier transform with modified Daniell smoothing[21]) was performed on each of these intervals. The vertical lines represent (from left to right) the 100-kyr, 41-kyr, 23-kyr and 19-kyr orbital periodicities. Note the increase in power at the 41-kyr periodicity at both sites after ~2.4 Myr.

At both sites whole-core volume susceptibility was measured at 3-5 cm intervals during the occupancy of the site. Between-hole correlations[18] using the susceptibility data were used to construct complete composite sequences. Age models were constructed using bio- and magnetostratigraphic data[19,20]. At Site 721 oxygen isotope data (W. L. Prell, unpublished data) were used to construct age-depth relations for the interval 0–1 Myr. The age models included as many data as possible within a series of straight-line segments. The record for Site 661 extends to 3.5 Myr and that for Site 721 extends to 3.2 Myr. Each time series was divided into 0.4-Myr intervals and spectral analysis[21] was performed on each interval. The resulting periodograms clearly demonstrated that variance was concentrated at orbital periodicities; minor adjustments were then made to some data (within their uncertainty limits) to improve the alignment of the peaks with the orbital periodicities.

Figure 2 shows the susceptibility time series and periodograms. The periodograms indicate a shift in spectral character at ~2.4 Myr. Before 2.4 Myr (2.4–3.5 Myr at Site 661; 2.4–3.2 Myr at Site 721), the data vary predominantly at the 23- and 19-kyr precessional periodicities. In each periodogram, the variance associated with this precession component significantly exceeds that associated with obliquity. After 2.4 Myr there is an increase in variance at the 41-kyr periodicity at both sites, and a corresponding reduction in variance at the 23- and 19-kyr periodicities. At Site 721, increased variance at the 41-kyr periodicity persists over the entire 0–2.4-Myr interval. At Site 661, the periodograms for the interval 0.0–0.8 Myr do not show variance at orbital frequencies; this may reflect an inadequate age model for this interval.

To examine the coherency between the susceptibility records and the orbitally driven insolation variations, the time series were filtered using a bandpass filter centred at 22 kyr, corresponding to orbital precession. The extracted component was then correlated ('tuned') to the calculated precession curve[1] using an inverse-correlation method[3,22]. Although coherency between the filtered susceptibility data and precession was not evident for Site 661, the filtered susceptibility data at Site 721

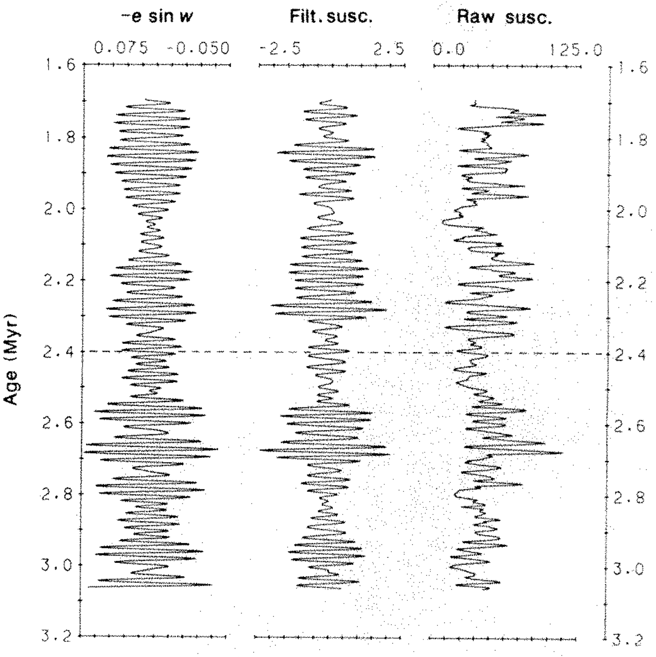

FIG. 3 Calculated orbital precession index (−*e* sin *w*), the 22-kyr filter of the susceptibility record and the raw susceptibility record for the 3.2–1.6-Myr interval at Site 721. The filtered data exhibited the modulated character of the precession record, and were therefore correlated ('tuned')[3] to the precession curve using an inverse-correlation method[22]. The 'tuned' age-depth data are within the uncertainties of the original age-depth model[23]. The coherency over the entire interval is 0.86; coherency from 3.2–2.4 Myr is 0.89.

exhibits the modulated character of the precessional curve (Fig. 3). Coherency over the 3.2-1.6-Myr interval is 0.84 and is even higher (0.86) from 3.2-2.4 Myr (ref. 23). Although the tuning procedure maximizes the correlation between the two signals, the coherency of the 400- and 100-kyr modulation envelopes is a real component of the susceptibility data. These data document the oldest and longest sequence demonstrating both power and coherency at the precessional band. We consider that these results demonstrate the strong orbital control of the susceptibility records at both sites, together with a shift from strong precessional forcing before, and strong obliquity forcing after, ~2.4 Myr. This raises the questions of what aspects of the relevant climate systems do the susceptibility records reflect; and what is the significance of the periodicity shift at 2.4 Myr?

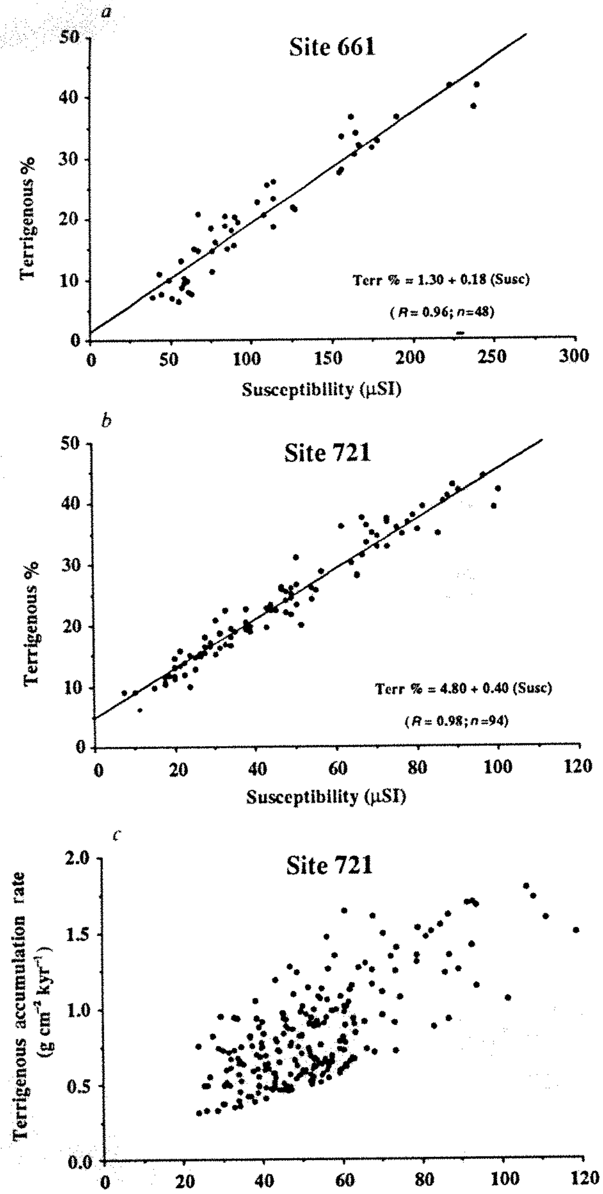

FIG. 4 *a, b,* Correlation between susceptibility and per cent terrigenous sediment for the Site 661 and Site 721 sediments. The terrigenous per cent values were calculated after sequentially removing calcium carbonate, opal and organic carbon components[23,24]. *c,* Correlation between susceptibility and terrigenous accumulation rate for the 2.5-2.7-Myr interval of Site 721. Because the susceptibility is highly coherent with orbital precession over this interval (see Fig. 3), detailed accumulation-rate data were calculated by phase-locking ('tuning') the raw susceptibility record to precession. The resulting accumulation-rate time series was combined with the relation shown in *b* to calculate the separate biogenic and terrigenous accumulation-rate data. The strong positive correlation indicates that susceptibility at Site 721 is reflecting variations in terrigenous accumulation, not biogenic dilution. The susceptibility-biogenic accumulation-rate plot, for which the correlation is poor, is not shown.

Terrigenous extraction procedures[24] were conducted on 94 samples from Site 721 (ref. 23) and 64 samples from Site 661. These data (Fig. 4*a, b*) show that susceptibility is an accurate proxy indicator of terrigenous content at both sites. Using the 'tuned' Site 721 susceptibility record to generate detailed age-depth relations for the 2.7-2.5 Myr interval, we converted the terrigenous concentrations to accumulation rates[23]. Figure 4*c* shows the strong positive correlation between susceptibility and terrigenous accumulation rate, indicating that the susceptibility variations are reflecting variations in terrigenous supply rather than biogenic dilution; by contrast, the correlation between susceptibility and biogenic accumulation rate was poor. A study of late Pleistocene terrigenous accumulation on the Owen Ridge[16] supports this conclusion.

The strong relationship between susceptibility and the concentration and accumulation rate of the terrigenous fraction, and the occurrence within this fraction of minerals indicative of an aeolian source[23], leads us to conclude that the Site 721 susceptibility record is a direct expression of variations in the wind transport of terrigenous material by the Asian monsoon. The interpretation of the Site 661 susceptibility record as a direct indicator of variations in terrigenous deposition is less certain. Fluctuations of deep water from Northern and Southern Hemisphere sources across the site, possibly at orbital periodicities, could have influenced its susceptibility record through variable calcite dissolution.

The predominance of 23- and 19-kyr variance in the susceptibility records before 2.4 Myr indicates that terrigenous deposition from the Asian, and possibly the African, summer monsoons was largely modulated by summer-season insolation variations due to precession. Atmospheric GCM experiments have demonstrated that monsoonal circulation is extremely responsive to precessional variations in local summer insolation, and that the response is approximately linear[14]. The marked increase in variance at the 41-kyr periodicity at ~2.4 Myr coincides with the initiation of major Northern Hemisphere glaciation[25], and GCM results and geological evidence indicate that this may reflect ice-sheet effects on the monsoon climate or the dust source regions. The increase in power at the 41-kyr periodicity is particularly significant because the late Pliocene marine $\delta^{18}O$ record varies almost purely at this periodicity[26]. GCM experiments have not unambiguously identified any direct effects of the large high-latitude Laurentide and Fennoscandian ice sheets on low-latitude hydrology[27], although there is geological evidence[10,28-30] indicating that African and Asian source areas may have experienced aridification during the last glacial maximum. Hence, the increase in 41-kyr power after 2.4 Myr may be linked to changes in the monsoon intensity or source-area climate due to the coeval expansion of the Eurasian or North American ice sheets, or both, which varied predominantly at this periodicity.

Our results clearly demonstrate the value of whole-core susceptibility logging for identifying potentially important time intervals deserving further investigation. Parallel mineralogical and grain-size studies will help to evaluate more fully the significance of susceptibility as an indicator of source-area climate and terrigenous deposition at both sites. More importantly, the integration of these data with on-going isotope and biotic assemblage studies will lead to a better understanding of the climatic origin of the 2.4-Myr shift and its relevance to other components of climate. □

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1. Berger, A. L. *Quat. Res.* **9**, 139-167 (1978).
2. Shackleton, N. & Opdyke, N. *Geol. Soc. Am. Mem.* **145**, 449-464 (1976).

3. Imbrie, J. *et al.* in *Milankovitch and Climate*, Vol. 1, 269–305 (Reidel, Hingham, Massachusetts, 1984).
4. Ruddiman, W. & McIntyre, A. *Bull. geol. Soc. Am.* **95,** 381–396 (1984).
5. Pokras, E. & Mix, A. *Quat. Res.* **24,** 137–149 (1985).
6. Prell, W. & Van Campo, E. *Nature* **323,** 526–528 (1986).
7. Mix, A. & Fairbanks, R. *Earth planet. Sci. Lett.* **73,** 231–243 (1985).
8. Barnola, J. M., Raynaud, D., Korotkevitch, Y. S. & Lorius, C. *Nature* **329,** 408–414 (1987).
9. Kent, D. V. *Nature* **299**, 538–539 (1982).
10. Bloemendal, J., Lamb, B. & King, J. W. *Paleoceanography* **3,** 61–87 (1988).
11. Doh, S.-J., King, J. W. & Lienen, M. *Paleoceanography* **3,** 89–112 (1988).
12. Pye, K. in *Aeolian Dust and Dust Deposits*, 76 (Academic, New York, 1987).
13. Ramage, C. S. *J. atmos. Sci.* **23,** 144 (1965).
14. Prell, W. L. & Kutzbach, J. E. *J. geophys. Res.* **92,** 8411–8425 (1987).
15. Sirocko, F. R. & Sarnthein, M. *NATO Adv. Res. Workshop* (Oracle, Arizona, 1987).
16. Clemens, S. & Prell, W. *Paleoceanography* (in the press).
17. Propero, J. M. *et al. Rev. Geophys. Space Phys.* **21,** 1607–1629 (1983).
18. Ruddiman, W. F., Cameron, D. & Clement, B. M. in *Init. Rep. DSDP* Leg 94, 615–634 (1986).
19. Ruddiman, W. F. & Sarnthein, M. in *Init. Rep. ODP* 108 A (1988).
20. Prell, W. L. & Niitsuma, N. in *Init. Rep. ODP* Leg 117 (in the press).
21. Bloomfield, P. *Fourier Analysis of Time Series: An Introduction* 258 (Wiley, New York, 1976).
22. Martinson, D. G., Menke, W. & Stoffa, P. *J. geophys. Res.* **87,** 4807–4818 (1982).
23. deMenocal, P., Bloemendal, J., King, J. W., Debrabant, P. & Chamley, H. in *Init. Rep. ODP* Leg 117 (in the press).
24. Rea, D. K. & Janecek, T. R. in *Init. Rep. DSDP* Leg 62, 653–659 (1981).
25. Shackleton, N. J. *et al. Nature* **307,** 620–623 (1984).
26. Ruddiman, W. F., Raymo, M. E. & McIntyre, A. *Earth planet Sci. Lett.* **80,** 117–129 (1986).
27. Rind, D. *J. geophys. Res.* **92,** 4241–4281 (1987).
28. Sarnthein, M. *Nature* **272,** 43 (1978).
29. Street, F. A. & Grove, A. T. *Quat Res.* **12,** 83 (1979).
30. Van Campo, E., Duplessy, J.-C. & Rossignol-Strick, M. *Nature* **296,** 56–59 (1982).

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Thermal entrainment by deflected mantle plumes

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HOTSPOTS are thought to result from deep mantle plumes, perhaps representing an ancient geochemical source isolated from the uppermost mantle. As they rise through the mantle, thermal plumes entrain surrounding material by thermal conduction, and small plumes or narrow plume conduits may consist of only a small fraction of original source material. Here we present new experimental results which show this effect dramatically for plume conduits that are deflected horizontally by shear flow in the mantle beneath lithospheric plates. Thermally entrained material can become concentrated at the centre of a plume (or surface hotspot trace) with original source material in the periphery. Also, the diapiric instability observed in chemical plume conduits is suppressed by thermal entrainment. Varying degrees of geochemical enrichment observed at hotspots such as the Galapagos Islands may result from thermal entrainment, implying that mantle plumes are driven by thermal (rather than compositional) buoyancy.

If volcanic hotspots such as Hawaii are caused by mantle plumes[1,2], then the nature of these buoyant plumes is constrained by some striking (although not necessarily consistent) features of hotspots. Hotspot plumes must (1) represent a deep mantle source that is relatively decoupled from lithospheric plate motions, (2) transport material that is geochemically enriched relative to the depleted upper mantle, and (3) represent a horizontal scale length (~10–100 km) of convection that is much smaller than that associated with plate motions.

Although it is not clear from observations whether mantle plumes are driven primarily by thermal or chemical buoyancy, a thermal origin at the core-mantle boundary is commonly assumed. Broad-scale, two-dimensional (sheetlike) thermal plumes have been modelled in many theoretical and experimental convection studies (refs 3-5, for example) in which lower-boundary-layer instabilities develop in response to basal heating. Experimental studies of narrow isolated plume conduits[6-10] have used chemically buoyant (non diffusive) plume material. Plume conduits exhibit a fascinating variety of behaviour including solitary conduit waves[10] and diapiric instability in horizontally deflected conduits[7,8]. Thermally buoyant conduits have not been studied experimentally, however, and thermal diffusion may be expected to dominate the dynamics of very narrow plumes. (Diffusion effects are not expected to be important in compositionally buoyant mantle plumes, because chemical diffusion rates in the mantle are generally thought to be much smaller than thermal diffusion rates.) Here we present results from preliminary experiments that demonstrate the important effects of thermal entrainment for plumes deflected horizontally by mantle shear flow beneath the lithospheric plates. These results are particularly applicable to the mixing of enriched and depleted mantle reservoirs by thermal plumes.

The thermal entrainment of surrounding material by rising hot diapirs has been demonstrated by Griffiths[11,12]. Figure 1 illustrates this process in which a boundary layer of heated surrounding material becomes entrained, because of its own thermal buoyancy, into the rising diapir. As the diapir rises, a torus or smoke ring of original source material forms, with entrained material forming the central core of the diapir for initial diapir Rayleigh numbers larger than about 12 (ref. 11). We show that this process also occurs in a horizontally deflected thermal plume as the plume conduit rises through the cold surrounding material. This can be envisioned by imagining that Fig. 1 represents not a cross-section of a spherical diapir, but instead a view along the axis of a cylindrical conduit that is rising through a fluid (see refs 13, 14). The superposition of fluid flow through the conduit and the bifurcated flow pattern shown in Fig. 1 will give rise to paired, helical particle paths as material ascends through the plume conduit, with thermal entrainment of heated surrounding material into the centre of the plume.

We observed this phenomenon using a simple experimental apparatus described in detail elsewhere[13]. A cylindrical Plexiglas tank (60 cm in diameter and 10 cm high) is filled with glycerol and fitted with a lid which is rotated slowly by an electrical motor, creating a simple (circular) shear flow in the tank beneath the lid or plate. The plume is fed gravitationally through a

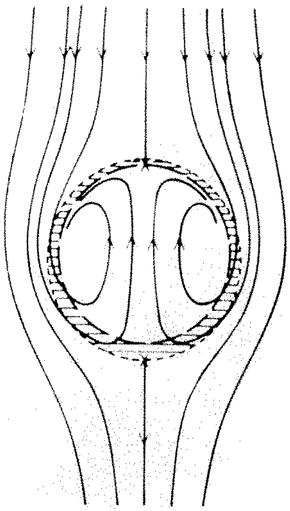

FIG. 1 Paths of fluid particles outside a hot, rising diapir[11]. Those particles that approach close to the diapir are heated by diffusion in the thin thermal boundary layer (hatched area), become buoyant, and are incorporated into the enlarging spherical vortex. In other words, they become entrained. Alternatively, this illustration can be taken as the view along the axis of a cylindrical plume conduit as in the experiments presented here.

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control valve from an elevated reservoir of glycerol containing a small amount of red dye. The plume source is a small hole (0.2-cm diameter) in the base of the tank, 16 cm from the lid rotation axis. The red glycerol is heated before it enters the tank by heating tape wrapped around the copper inlet pipe. The temperature of the thermally buoyant plume material is monitored by a small thermocouple just below the inlet hole.

The photographs of Fig. 2*a*, *b* show side and top views of a horizontally deflected plume that exhibits strong thermal entrainment. The red dye (dark material) represents the original plume material injected at the base of the tank, and it forms two stripes along the outside of the plume as seen in top view. The central core of the plume consists of entrained material. The plume remains largely a circular cylindrical conduit, expanding as it rises in the tank. The relevant experimental parameters are: tank-fluid kinematic viscosity $\nu_{\mathrm{t}} = 1.5\ \mathrm{cm}^2\ \mathrm{s}^{-1}$, upper-plate velocity above the plume source $u_0 = 0.23\ \mathrm{cm\ s}^{-1}$, temperature contrast between plume source and tank fluid $\Delta T = 50\ ^{\circ}\mathrm{C}$, density contrast $\Delta\rho = 0.018\ \mathrm{g\ cm}^{-3}$. (The glycerol in this experiment absorbed some water from the air so that its viscosity was about a factor of four less than that of pure glycerol.) In the case shown, the Prandtl number is sufficiently large ($\mathrm{Pr} = 320$) and the Reynolds number (for the rise of the conduit through the tank fluid) sufficiently small ($\mathrm{Re} = 0.01$) to assure dynamical similarity to creeping flow in the mantle. The initial plume diameter is 0.3 cm and it expanded to >1.0 cm just beneath the tank lid. (We note that thermal expansion of plumes occurs whether or not there is significant horizontal deflection[15].) This represents about a factor of ten increase in plume cross-sectional area, giving an order of magnitude decrease in average temperature contrast across the plume. The half-angle expansion ratio of the conduit[11] is approximately constant at $\varepsilon = 0.049$. (ε is one-half the increase in plume diameter per unit plume-rise distance.) The original plume material accounts for only about one-tenth of the thermally expanded plume volume near the tank lid.

We can scale this experiment to plumes in the Earth's mantle by defining a conduit Rayleigh number $\mathrm{Ra} = g\Delta\rho V/\kappa\nu_{\mathrm{t}}$ where g is the gravitational acceleration and κ is the thermal diffusivity of the tank fluid. The Rayleigh number is simply a dimensionless thermal buoyancy, and it is not intended to represent the vigour of mantle convection. V is defined[13] as an effective 'conduit element' volume based on the initial conduit diameter d so that $V = 2.9\pi d^3/8$. (The factor $2.9\pi/8$ is somewhat arbitrary[13] and of no consequence with respect to the following scaling arguments.) Thus, the conduit Rayleigh number for the plume of Fig. 2*a*, *b* is $\mathrm{Ra} = 24$. This can be scaled to the mantle as follows: for a plume of reasonable density contrast $0.02\ \mathrm{g\ cm}^{-3}$ ($\Delta T \approx 200\ ^{\circ}\mathrm{C}$), diameter 50 km and thermal diffusivity $\kappa = 0.01\ \mathrm{cm}^2\ \mathrm{s}^{-1}$, and for a mantle viscosity of 10^{22} P, we obtain a similar Rayleigh number $\mathrm{Ra} = 29$. The plume-conduit Rayleigh number increases with the cube of the plume diameter and decreases with the viscosity of the surrounding fluid, and neither of these parameters is well constrained for mantle plumes. The experimental results shown are applicable, for example, for mantle-plume diameters of ~5–80 km with background mantle viscosities of $\sim 10^{19}$–10^{23} P, respectively. Clearly, the range of conduit Rayleigh numbers in our experiments may apply to real mantle plumes.

Another important parameter is the upper-plate velocity, which is $u_0 = 0.23\ \mathrm{cm\ s}^{-1}$ here. This can be scaled to lithospheric plate or mantle shear-flow velocity using the Peclet number (dimensionless velocity). The Peclet number is defined as $\mathrm{Pe} = u_0 L/\kappa$, where the appropriate scale length is the tank fluid depth $L = 10$ cm. For the experiments shown, $\mathrm{Pe} = 290$. Scaling to the Earth's mantle depth $L_{\mathrm{m}} = 3{,}000$ km and using $\kappa = 0.01\ \mathrm{cm}^2\ \mathrm{s}^{-1}$, we obtain the characteristic mantle shear velocity $u_{\mathrm{m}} = 0.32\ \mathrm{cm\ yr}^{-1}$. This is appropriate either for a very slow litho-

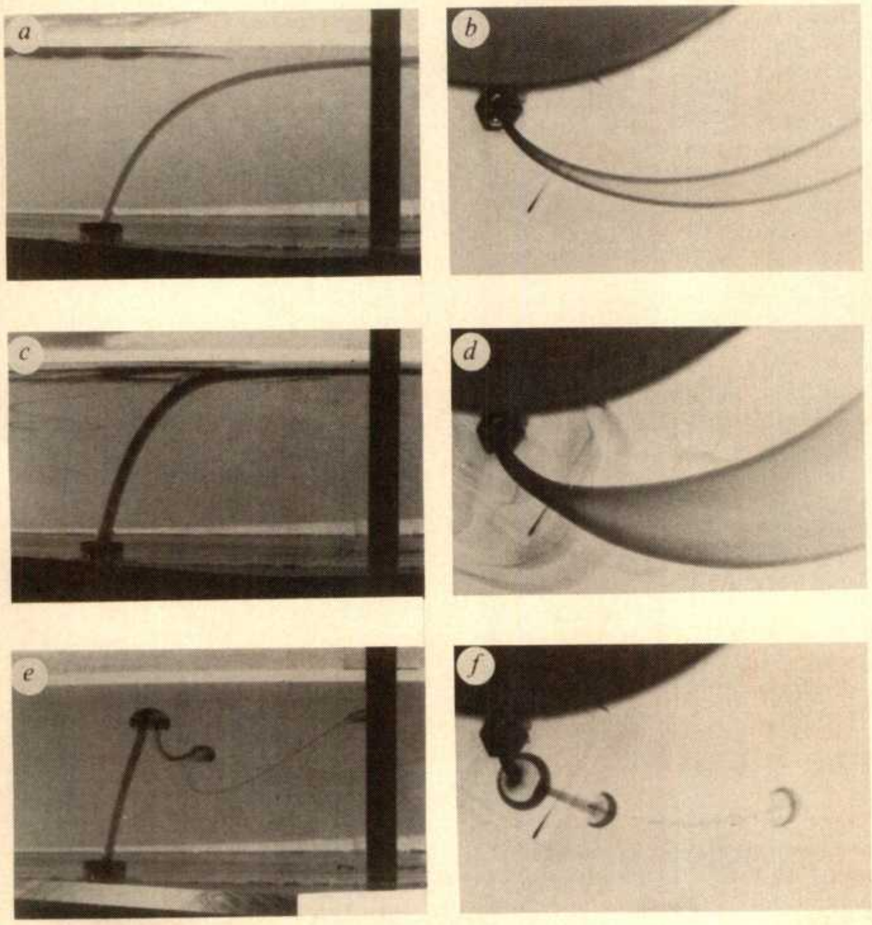

FIG. 2 Side (*a*, *c*, *e*) and top (*b*, *d*, *f*) views of the plume experiments. In *a*, *c* and *e* the dark vertical bar on the right-hand side is the lid rotation axis. The 1-cm-thick lid is the light horizontal band just above the point where the red (dark), thermal plume becomes horizontal. In *b*, *d* and *f* the drive wheel appears dark and unfocused in the upper left-hand corner. The lid rotation is counter-clockwise along the curvature of the dark plume trace. The faint grey cloud above the plume source in *d* is diffused plume material from previous experiments that has been advected around the cylindrical tank.

spheric plate or as a characteristic velocity in the Earth's deep mantle. (The relatively low-viscosity upper mantle, or asthenosphere, beneath the plates indicates deep-mantle flow velocities about an order of magnitude less than typical plate velocities[16].) Larger upper plate or shear-flow velocities, with other parameters constant, will result in more horizontal deflection[13] and even stronger thermal entrainment effects, especially for higher Rayleigh numbers.

Based on experiments with hot rising diapirs[11], we expect significant thermal entrainment for Rayleigh numbers as high as ~10,000. Figure 2*c*, *d* shows a larger plume (higher plume feed rate) under experimental conditions that are otherwise the same as for Fig. 2*a*, *b*. The initial plume diameter is 0.6 cm, yielding a higher conduit Rayleigh number of 192. The measured expansion ratio is $\varepsilon = 0.027$, which is smaller than for the previous experiment ($\mathrm{Ra} = 24$; $\varepsilon = 0.049$). This is expected, because a higher Rayleigh number implies a smaller thermal boundary-layer thickness relative to the conduit diameter. The two experiments here indicate a somewhat smaller decrease in ε with increasing Rayleigh number than for diapirs[11]. For the higher-Rayleigh-number experiment in Fig. 2*c*, *d* the original plume material constitutes only about 35% of the volume of the plume as it encounters the tank lid.

Returning to Fig. 2*a*, we note that in this experiment and for other thermal-plume conduits deflected to almost horizontal orientations (data not shown), we never observed any diapiric-conduit instability. Apparently, plumes of modest Rayleigh number are stabilized by thermal entrainment of surrounding material. This is an important observation, because diapiric instability in deflected plume conduits (observed in laboratory models of chemical plumes) has been suggested as the cause for individual volcanic centres along hotspot tracks[7] and for sea-floor swells[9]. Thermal plumes probably remain stable because of the ever expanding thermal-conduit width and because of the reduced viscosity contrast across the plume. (At a temperature contrast of 50 °C, the plume interior is initially about an order of magnitude less viscous than the surrounding fluid.) A detailed stability analysis, similar to Whitehead's for chemical plumes[17], is thus warranted for thermal plumes.

Figure 2*e*, *f* show another new observation. Thermal conduit waves (or solitons) are induced by three sudden increases in conduit feed rate. These three blobs show the thermal entrainment expected[11], but the effect of horizontal deflection causes a strongly asymmetrical pattern in the original source material as the blobs rise. If such hot blobs are considered as hotspot sources, then the original (enriched mantle) plume material would be least diluted in the 'downstream' direction of plate motion with the most strongly diluted material toward the 'upstream' side of the volcanic centre.

The most conspicuous evidence for the effects we have discussed is found in the geochemistry of basaltic lavas from the Galapagos hotspot, for which Geist *et al.*[18] have described a puzzling open-horseshoe pattern of strontium isotope enrichment. This pattern is thought to reflect source heterogeneity, because variations in trace-element concentrations, which reflect differences in melting and fractionation processes, are not well correlated with the variations in the isotope ratios. Geist *et al.*[18] proposed that this source heterogeneity resulted from thermal entrainment by a persistent hot diapir beneath the lithosphere, with a depleted (upper mantle) core surrounded by an enriched (lower mantle) torus. The least-enriched basalts are found toward the downstream (east) side of the Galapagos archipelago, however, which is just the opposite pattern shown in our experiments (Fig. 2*f*).

There are other problems with the single-diapir model for the Galapagos hotspot. First, a solitary diapir would obviously not remain stationary beneath the lithosphere, but would be advected along with the plate motion. This is inconsistent with the fact that the volcanism propagates westward relative to the overlying oceanic plate at the expected rate (~5 cm yr^{-1}) for a fixed mantle plume. The second problem is that the continuity of the Cocos and Carnegie Ridges (twin hotspot tracks formed while the Galapagos spreading centre coincided with the Galapagos hotspot) requires long-lived continuity of the Galapagos plume before ~5 Myr.

We suggest an alternative model which might better explain the Galapagos isotope data. Thermal entrainment by a continuous, horizontally deflected plume would result in a spreading V pattern (see Fig. 2*b*, *d*), with the least-enriched (upper mantle) source material toward the downstream side of the hotspot track (as observed). Scaling from the experiment in Fig. 2*d* to the ~150-km-wide plume top needed to explain the width of the Galapagos platform, we find a comparable conduit Rayleigh number of ~200 for an upper-mantle viscosity of 10^{22} P and an original plume excess temperature of ~250 °C. Scaling to the upper-mantle depth of 600 km, a comparable Peclet number of ~300 requires an average upper-mantle shear velocity of ~1-2 cm yr^{-1}. As in Fig. 2*d*, such a plume would consist of only about 35% original source material, with a final temperature excess beneath the lithosphere of ~100 °C. The parameters we have assumed are not well constrained, and we emphasize that our model is speculative. A major field effort is presently underway in the Galapagos to test these ideas, and a more complete set of laboratory experiments will be reported elsewhere.

Our experimental parameters emphasize the true plume width and maximize the degree of dilution or entrainment. Differing degrees of thermal entrainment (or horizontal deflection) may explain some of the variations in the degree of geochemical enrichment observed for different hotspots[19]. Application of these ideas to geochemical data from hotspot tracks such as the Galapagos may help determine whether or not mantle plumes are driven primarily by thermal or compositional buoyancy. ☐

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1. Morgan, W. J. *Geol. Soc. Am. Mem.* **132,** 7-32 (1972).
2. Morgan, W. J. in *The Sea* (ed. Emiliani, C.) 443-487 (Wiley, New York, 1981).
3. Parmentier, E. M. *et al. J. geophys. Res.* **80,** 4417-4424 (1975).
4. Yuen, D. A. & Schubert, G. *J. geophys. Res.* **81,** 2499-2510 (1976).
5. Olson, P. *et al. Nature* **327,** 409-413 (1987).
6. Whitehead, J. A. & Luther, D. S. *J. geophys. Res.* **80,** 705-717 (1975).
7. Skilbeck, J. N. & Whitehead, J. A. *Nature* **272,** 499-501 (1978).
8. Olson, P. & Singer, H. A. *J. Fluid Mech.* **166,** 511-531 (1985).
9. Olson, P. & Nam, I. S. *J. geophys. Res.* **91,** 7181-7191 (1986).
10. Whitehead, J. A. & Helfrich, K. R. *Nature* **336,** 59-61 (1988).
11. Griffiths, R. W. *J. Fluid Mech.* **166,** 115-138 (1986).
12. Griffiths, R. W. *Phys. Earth planet. Inter.* **43,** 261-273 (1986).
13. Richards, M. A. & Griffiths, R. W. *Geophys. J. R. astr. Soc.* **94,** 367-376 (1988).
14. Griffiths, R. W. & Richards, M. W. *Geophys. Res. Lett.* **16,** 437-440 (1989).
15. Loper, D. E. & Stacey, F. D. *Phys. Earth planet. Inter.* **33,** 304-317 (1983).
16. Davies, G. F. *Geophys. J. R. astr. Soc.* **49,** 459-486 (1977).
17. Whitehead, J. A. *Geophys. J. R. astr. Soc.* **70,** 415-433 (1982).
18. Geist, D. J. *et al. Nature* **333,** 657-660 (1988).
19. Zindler, A. & Hart S. *A. Rev. Earth planet. Sci.* **14,** 493-571 (1986).

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Gravity changes as a precursor to volcanic eruption at Poás volcano, Costa Rica

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MONITORING of gravity changes at active volcanoes has considerable potential for understanding magma-chamber dynamics and for detecting eruptive precursors. The technique essentially aims to identify changes in sub-surface mass or density corresponding to departures from the free-air gradient (FAG) or Bouguer-

corrected (terrain-corrected) FAG (BCFAG), respectively[1,2]. Such gravity changes, of the order of 10–100 μgal, have been reported previously through eruptive periods[3–6]. Here we present a ten-year data set measured at Poás volcano, Costa Rica, in which gravity increases in 1987–1989, relative to both the FAG and the BCFAG, preceded the ash-cloud eruptions of April–May 1989. These results constitute the first unambiguous detection of gravity changes as a precursor to eruption, and we interpret them in terms of the intrusion of an ascending, relatively dense magma body.

The last major eruption at Poás was in 1953, when phreatic activity culminated in the growth of a 30-m-high pyroclastic cone and the formation of an inner crater, both within the old summit crater. Since then, a hot crater lake has absorbed any fluctuations in heat output[7] from the magma feeder pipe by changing in volume, temperatuture and evaporation rate. Microgravity monitoring at Poás began in 1979 and was intensified to include more stations and some elevation control in 1985 (refs 8, 9). Compared with a reference point 2 km south of the active crater, we observed no significant gravity change at the three crater rim or eight flank stations between 1979 and 1983, although intensive monitoring over a 43-day period in 1983 did reveal cyclic variations[8]. We interpreted this phenomenon, observed again in 1985 during more detailed monitoring of sixteen crater stations, in terms of cyclic variations in the degree of vesiculation within the summit magma feeder pipe[9]. Since 1985, we have observed unidirectional gravity changes at crater stations on and around the 1953 cone. Relative elevation measurements, using a theodolite with an infrared electronic distance-measuring device yielding a precision of ∼2 cm, have been made since 1987 so that we could examine the possibility of sub-surface mass changes.

To observe long-term gravity changes, we average the data for each period of observation (typically several days), thus smoothing out any short-period cyclic variations. Uncertainties (which we determine by the reproducibility of difference measurements) are, at worst, up to 40 μgal for data obtained before March 1987, and are <25 μgal after this date because of the use of electronic readout and many more repeat readings[10]. Although there are a few erratic readings in the data set, nevertheless the mean difference between the two instruments is only 28 ± 26 μgal, similar to the uncertainty analysis above (and in ref. 10). The gravity variations relative to the reference point observed between 1979 and 1989 at crater-rim stations (R2A and R7, for example) are irregular and do not show a clear trend (Fig. 1). At crater-bottom stations E1, E3 and D1 (adjacent to the 1953 cone), however, relative gravity has clearly increased since March 1987. Indeed, total changes at the three stations on the 1953 pyroclastic cone (Fig. 1 inset) have been of the order of 200 μgal between 1985 and 1989. On the north side of the crater lake, only station D3 has experienced unambiguous decreases in the relative gravity from 1985 onwards. Because the gravity changes at stations E5–D1 (Fig. 1) are more than several times the size of the uncertainties involved, and two Lacoste and Romberg meters have been used (since 1987), the increases are undoubtedly genuine.

To evaluate the Bouguer-corrected free-air effect on gravity we assume a typical density. Measurements of surface rocks in the crater area yield densities of ∼2.67 Mg m^{-3} (for lavas) falling to ∼1.17 Mg m^{-3} for pyroclastic ashes, giving an estimated average of ∼2.2 Mg m^{-3} for the shallow and surface materials. For a given elevation change, assuming the usual free-air gradient and no density change, gravity will vary according to

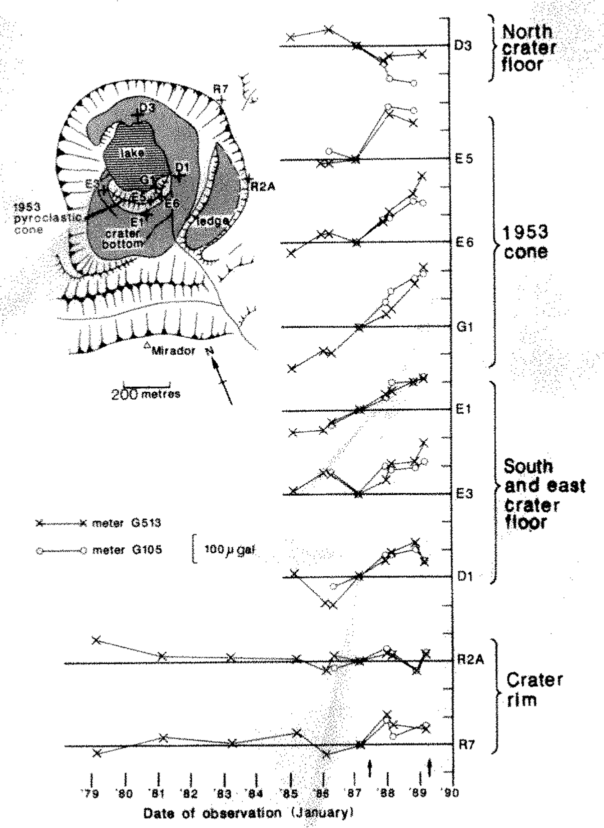


FIG. 1 Gravity variations using Lacoste and Romberg gravity meter G513 and (since 1987) G105 are expressed relative to the value at a reference point on the volcano flank for two crater-rim and seven crater-bottom stations with good gravity and elevation control. Each data point represents the average value obtained during 3–10 survey days. For details of errors, see text. The two arrows at the bottom of the figure indicate periods of dramatic change in surface activity. Note that if crater-bottom (or rim) gravity data were affected by the recent decrease in lake level, a relative decrease in gravity would be observed (as at D3, which seems to be remote from the centre-of-mass increase). Because all stations except D3 experience a gravity increase, the effect of the falling lake and water table is either negligible or at least small—except on the northern lake side—compared with another opposite effect causing a gravity increase. The inset shows station locations.

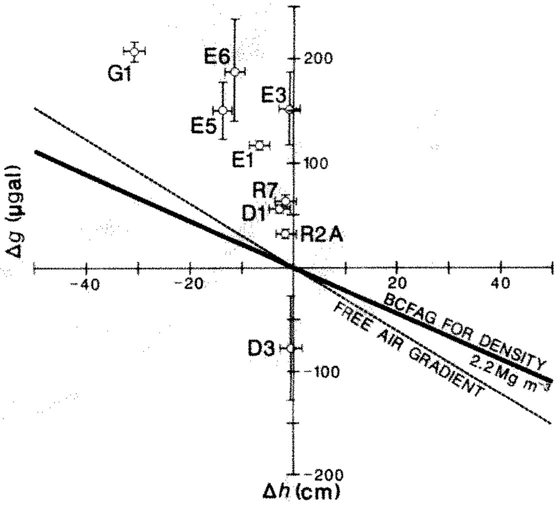


FIG. 2 Plot of the change in gravity against the change in elevation with the theoretical FAG and BCFAG marked for reference[1,2]. The mean gravity change as recorded by the two instruments is plotted with error bars reflecting the difference between the two meters for the 1987–1989 comparison (see Fig. 1, where the two meters differ by 28 ± 26 μgal). The stations read most frequently, which are G1, D1, E1 and R2A, have the smallest error bars on this basis. Errors in elevation differences are estimated as ±2 cm. An overall gravity increase is observed between March 1987 and March 1989 at all crater stations, except D3, even after account is taken of deflation, because all points plot above the FAG and BCFAG lines. The residual gravity increase over this period beneath stations E1, E3, E5, E6 and G1 is 100–150 μgal; see text for interpretation of the corresponding shallow mass increase.

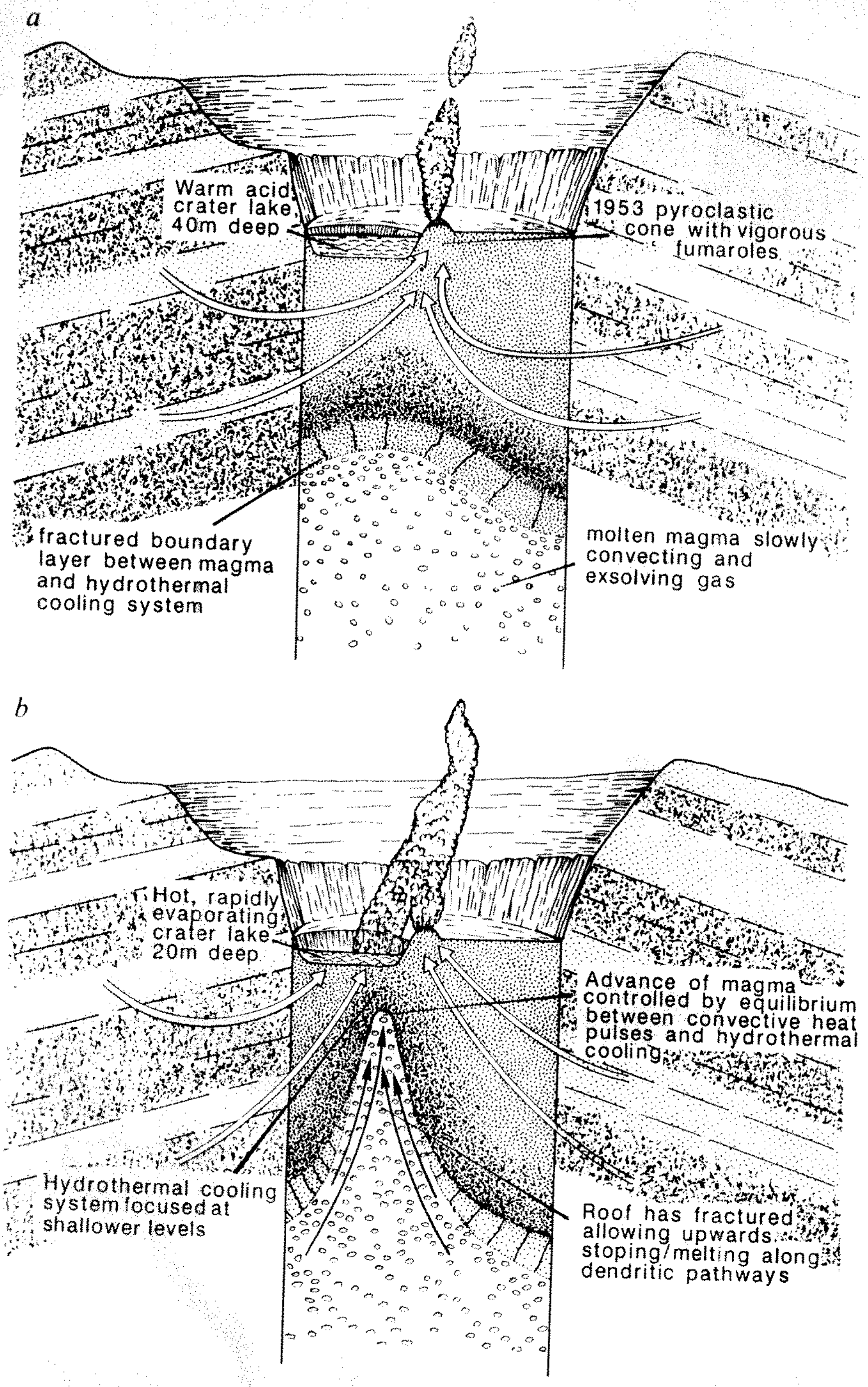
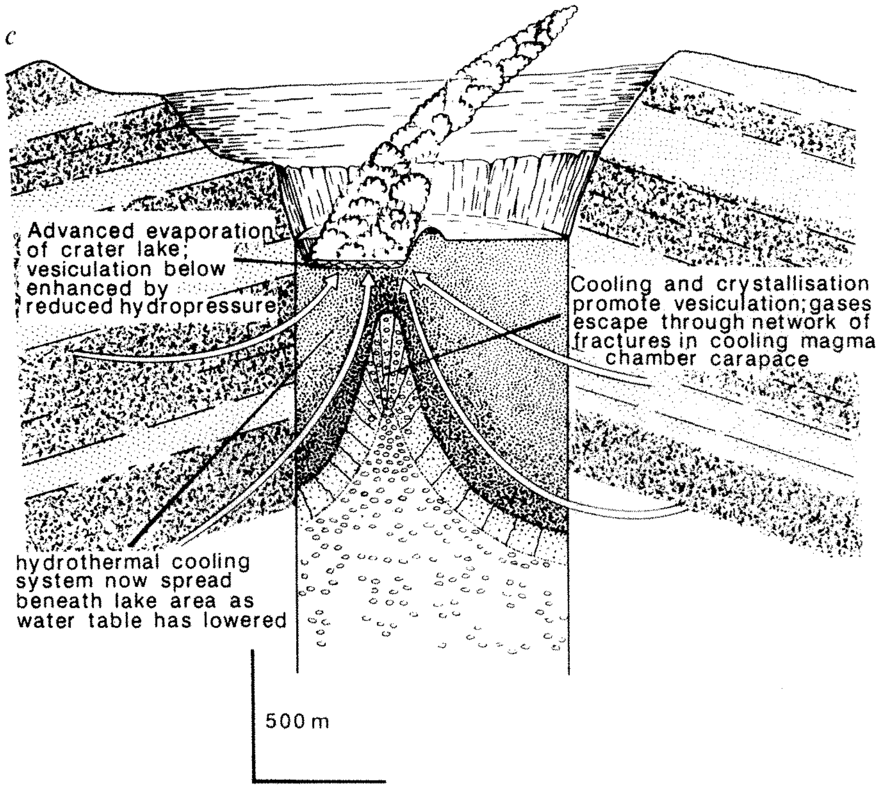

a
Warm acid crater lake, 40m deep
1953 pyroclastic cone with vigorous fumaroles
fractured boundary layer between magma and hydrothermal cooling system
molten magma slowly convecting and exsolving gas
b
Hot, rapidly evaporating crater lake 20m deep
Advance of magma controlled by equilibrium between convective heat pulses and hydrothermal cooling
Hydrothermal cooling system focused at shallower levels
Roof has fractured allowing upwards stoping/melting along dendritic pathways

FIG. 3 Schematic north–south cross-sections of Poás volcano summit and postulated evolution (*a–c*) of the magma chamber and its upper boundary layer. Within this layer, heat is removed by the hydrothermal steam-water system from the frozen, fractured cap of the partially molten magma body, initially at ~500 m depth. *a* shows the steady-state 1985 model developed in refs 7 and 16; by 1987 (*b*) we propose that the boundary layer had fractured extensively, allowing fingers of magma to ascend to a new equilibrium level where their upwards progress was halted by the increasingly efficient cooling system; in late 1988 (*c*), as pressure on the cooling magma system reduced and crystallization advanced, the collection of volcanic gases within the magma and their discharge through an extended fractured boundary layer is envisaged.

the BCFAG. The predicted BCFAG for this density is −216 μgal m^{-1} and we attribute (to a first approximation) observations that fall away from this line to sub-surface density changes[1-6]. This simple model requires change to occur only along an extensive horizontal slab; more complicated regions of dilatation/contraction do not follow a linear Δg–Δh relationship[2]. We present data for March 1987–March 1989 in Fig. 2; although there is some scatter, it is clear that overall subsidence of the crater rim and bottom region (maximum of 31 cm, measured relative to the Mirador benchmark shown in Fig. 1 inset) was accompanied by an 'excess' gravity increase. A net gravity increase above the BCFAG during a period of elevation decrease reflects an overall sub-surface density increase and a net gravity increase above the FAG reflects an overall mass increase. The excess gravity increase above both the BCFAG and FAG is smallest on the crater rim and largest on the pyroclastic cone, which is also the region of maximum deflation (especially at station G1). For stations E1, E3, E5, E6 and G1 the excess gravity increase above the FAG is 100–150 μgal (Fig. 2).

Records of historical activity at Poás[11,12] show that the recent geysering and disappearance of the lake, culminating in ash eruptions (as in 1989) are following a similar pattern to the rather poorly documented 1828, 1834, 1910 and 1953 eruptions. A warm (~40 °C) acidic crater lake remains apparently quite stable for a number of years, then shrinks dramatically and disappears during phreatic activity, which often culminates in a relatively minor ash-pyroclastic eruption. Before the present activity the Poás crater lake had maintained dynamic equilibrium since the late 1970s (refs 7, 13), however the lake level began to fall slowly in 1982 and by March 1989 it had fallen by a total of 40 m, 32 m of which occurred between March 1987 and March 1989 (refs 7, 14). Changes in the style of surface activity are marked on Fig. 1 at June 1987 when geyser eruptions began[14] and April 1989 when a 2-km-high ash cloud (reworked material) was erupted periodically until the crater lake was


c
Advanced evaporation of crater lake; vesiculation below enhanced by reduced hydropressure
Cooling and crystallisation promote vesiculation; gases escape through network of fractures in cooling magma chamber carapace
hydrothermal cooling system now spread beneath lake area as water table has lowered
500 m

re-established in mid-May. Interestingly, we observe the most significant gravity changes at stations on the 1953 cone both before 1987 and during the 1987–1989 period. Elevation data are not available before 1987, but gravity data for most crater stations show a similar trend from 1985 onwards, so assuming that elevation changes were no greater than those since 1987, the magnitude of the excess gravity increase over the four-year 1985–1989 period is estimated at ~200 μgal over an area of $\sim 10^4\ \mathrm{m}^2$. By applying Gauss' theorem and assuming the new mass has a density 2.67 $\mathrm{Mg\ m}^{-3}$ (the same as the erupted lava) we calculate a total sub-surface mass increase of $\sim 10^8$ kg.

An overall density increase beneath the crater area of volcanoes like Poás is usually interpreted[1,3] either in terms of magma devesiculation, or the collapse of voids, or the ascent of relatively dense magma into low-density agglomerates and ashes. In view of the 1987–1989 deflation, void collapse is a possibility, but because the power output at Poás almost doubled between 1985 and 1988 (ref. 7), we prefer a model involving upwards ascent of magma. As the 1985–1987 gravity increase is only observed at stations on and close to the 1953 cone, and thereafter the effect is largest at these stations, the depth of the causative intrusion using a half-width approximation must be shallow, probably <200 m. The intrusion is not necessarily directly beneath the cone, because gravity increases more to the north (that is, from E1 to E6 to G1); it may be centred on the fault at the north side of the cone or even further north beneath the lake (where we have no data). Given the steady-state starting situation in Fig. 3*a* (refs 7–9), we suggest that a small dendritic intrusion rose (Fig. 3*b*) from a region of partial melt ~500 m beneath the crater[9] through cracking of the magma-chamber roof zone[15], perhaps because hydrothermal cooling was unable to extract heat at the rate being supplied from below[7]. As the magma advanced it drove off ground waters producing elevated fumarole temperatures (500 °C measured on the cone in 1985). As the lake level fell because of more rapid evaporation, the water table would become lower (which may account for the gravity decreases observed at station D3) and eventually the fumaroles on the cone would have cooled and almost disappeared as heat output switched to the crater lake (the bottom of which is some 30 m below the base of the cone; Fig. 3*c*). As the cone cooled from ~500 °C (1985) to ~100 °C (1988), it would be expected to contract. The cone consists of vesiculated, blocky and altered andesite lava, with a thermal expansivity of $\sim 5 \times 10^{-5}\ {}^\circ\mathrm{C}^{-1}$, so a drop of 400 °C could easily result in 1% contraction of the 30-m-high cone (note the 31-cm deflation at station G1, Fig. 2). Deflation would also accompany the fall in water vapour pressure within the edifice as the water table fell below the base of the cone to its early 1989 position just above the lake bottom (Fig. 3*c*).

This is the first unambiguous record of gravity changes occurring before an eruption (in this case, the April–May 1989 ash cloud); it highlights the potential of microgravity monitoring as a predictive technique in the surveillance of active volcanoes. The power output at Poás is calculated to have increased from ~160 ± 50 MW to ~265 ± 100 MW between 1985 and 1988 (ref. 7) and this is consistent with the injection of shallow magma beneath the crater lake (Fig. 3*b*). The possibility of a major pyroclastic eruption involving juvenile material, resulting from the advanced buildup of magmatic gas pressure[8] in the area of the pyroclastic cone and lake, cannot be ruled out. ☐

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1. Rymer, H. *Proc. Kagoshima Int. Conf. Volcanoes*, 358–361 (National Institute for Research Advancement, Tokyo, and Kagoshima Prefectorial Government, Kogoshima, 1988).
2. Savage, J. C. *J. geophys. Res.* **89,** 1945–1952 (1984).
3. Rymer, H. & Brown, G. C. J. Volcan. geotherm. Res. **27,** 229–254 (1986).
4. Eggers, A. A. *J. Volcan. geotherm. Res.* **33,** 201–216 (1987).
5. Dzuirisn, D. *et al. J. Volcanol. geotherm. Res.* **7,** 241–269 (1980).
6. Jachens, K. C. & Eaton, G. P. *J. Volcanol. geotherm. Res.* **7,** 225–240 (1980).
7. Brown, G. C. *et al. Nature* **339,** 370–373 (1989).
8. Rymer, H. & Brown, G. C. *Nature* **311,** 243–245 (1984).
9. Rymer, H. & Brown, G. C. *Bull. volcan.* **49,** 389–398 (1987).
10. Rymer, H. *Geophys. J. R. astr. Soc.* **97,** 311–322 (1989).
11. Vargas, C. A. *Antologia: el volcan Poás* (Univ. Estatal a Distancia, San Jose, Costa Rica, 1979).
12. Krushensky, R. D. & Escalante, G. *Bull. volcanol.* **31,** 75–84 (1967).
13. Brantley, S. L., Borgia, A., Rowe, G., Fernandez, J. F. & Reynolds, J. F. *Nature* **330,** 470–472 (1987).
14. Scientific Event Alert Network **12,** 7–8 (1987); **14,** 15–16 (1989).
15. Casertano, L. *et al. Geofis. Int.* **24,** 315–332 (1985).
16. Rymer, H., Morales, L. D. & Brown, G. C. *Revista Geologico de America Central* **6,** 75–102 (1987).

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A helium isotope transect along the Indonesian archipelago

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HELIUM isotope studies[1,2] have identified the Banda arc section of the Indonesian archipelago as the only circum-Pacific volcanic arc with $^3He/^4He$ ratios significantly lower than the common ratio in mid-ocean-ridge basalts (MORB). In eleven of twelve circum-Pacific arcs, MORB-like $^3He/^4He$ ratios show that helium in these volcanic terrains is dominantly primordial helium from the mantle wedge above the sinking slab, rather than radiogenic helium from subducting oceanic crust or sediments. Only in the Banda arc have significantly lower ratios been observed, corresponding to as much as 80% radiogenic helium from subducting continental material[2]. We now report $^3He/^4He$ measurements along a 1,800-km section of the Sunda arc, a region of underthrust oceanic lithosphere, showing MORB-like ratios from western Java to a surprisingly sharp transition zone centred on Lomblen Island, where the low ratios of the Banda arc begin. Our results confirm the prediction[2] that Sunda arc volcanoes should have MORB-like $^3He/^4He$ ratios, with a well-defined transition to the much lower Banda arc ratios near Lewotolo volcano, and demonstrate a remarkable linkage of helium isotope variations with tectonic processes not readily discernible with trace elements or other isotopes.

The Indonesian arc system is a study in contrasting tectonics[3-5]. In the Sunda arc on the west, oceanic lithosphere of the Austral-Indian plate is subducting northward along the Java Trench under the continental margin of the Eurasian plate. On the east the contiguous Banda arc is a reciprocal system with northward subduction of the Australian continental margin, also on the Austral-Indian plate, under the Banda Sea. Both arcs include typical arc volcanics and high-potassium calc-alkaline lavas[6-8]. The Sunda arc however, along 2,000 km from western Java to Lomblen, is punctuated at ~650-km intervals by leucite-bearing potassium-rich volcanoes[9], such as Muriah on Java[10], Sangenges and Soromundi on Sumbawa[11] and Batu Tara north of Flores[9]. Strontium and neodymium isotope data[9-16] are the primary discriminants for the two arcs: Sunda arc lavas lie within the mantle-array region defined by other island arcs, whereas Banda arc lavas are displaced toward more radiogenic Sr and Nd ratios, indicating mixing with sialic crustal material[17]. The isotope data and tectonic interpretation are thus consistent with one another and indicate that the Banda arc is the only volcanic island arc and continent collision zone in which underthrusting of continental crust is occurring.

We measured the $^3He/^4He$ ratios of volcanic gases from nine sites at five active volcanoes on Java, Flores and Lomblen: helium in phenocrysts from recent lavas was measured at three other volcanoes on Bali, Sumbawa and Batu Tara (Komba). In Fig. 1 and Table 1 we give the $^3He/^4He$ ratios at each site; data from our previous study[2] of Banda arc volcanoes are also included. Lewotolo volcano was resampled (at different sites) with identical results. The most important results show that the Sunda arc volcanoes along a 1,500-km section from western Java to central Flores (Tankguban Parahu, Merapi, Batur, Sangeang Api and Keli Mutu) have $^3He/^4He$ ratios of $R/R_A = 5.7\text{-}8.2$ (where R_A is the $^3He/^4He$ ratio of air), almost exactly the range in the circum-Pacific arc systems studied previously[2]. Compared to the mean MORB ratio ($R/R_A = 8 \pm 1$) and radiogenic or 'crustal' He ($R/R_A < 0.02$), these volcanic ratios correspond to a mantle (MORB) helium fraction of 70-100%. The Java-Bali-Sumbawa-Flores $^3He/^4He$ ratios are thus very different from the Pantar and Banda arc ratios ($R/R_A = 1.1\text{-}1.7$) in which the helium is in every case more than 80% of radiogenic origin. Because the Banda arc is unique among volcanic-arc terrains in emitting predominantly radiogenic helium, and is unique in having collided with, and been underthrust by, continental crustal material, it is evident that the Banda arc helium is derived from the subducting Australian crustal material.

Between the low R/R_A volcano Sirung on the island of Pantar and the first active volcano of the Banda arc, Damar, a 500-km inactive segment has been interposed by completion of the suture between the Banda arc and the Australian continental margin and cessation of active underthrusting[3,4]. The transition from mantle to radiogenic helium emission is not in the Banda arc itself, nor is it across the volcanic quiet segment: the actual $^3He/^4He$ transition zone is the narrow 200-km section between Egon volcano on the west, at the narrowest width of eastern Flores, and Sirung volcano on the east, at the southern tip of Pantar[18]. These two volcanoes have the lowest $^3He/^4He$ ratios in the arcs ($R/R_A = 1.25$ and ~ 1.1), but between them, at nearly the midpoint of the transition zone, Lewotolo volcano within the arc and Batu Tara 53 km to the north in the Banda Sea have identical elevated $^3He/^4He$ ratios of 3.4 and 3.6 R_A. The $^3He/^4He$ structure of this zone is thus complex and deserves further study.

In Fig. 2 we compare the $^{87}Sr/^{86}Sr$ profile (see refs 6, 10 and 19) and the $^3He/^4He$ ratios along the 2,800-km section from west Java to Serua at the eastern extremity of the Banda arc. The sharp drop in $^3He/^4He$ ratio at the transition zone (6.0 R_A to 1.25 R_A in only 70 km from Keli Mutu to Egon) is in marked contrast to the approximately linear increase in $^{87}Sr/^{86}Sr$ over the ~750-km section from Bali to Egon, and even more so for the 1,500-km increase from Bali to Damar. That is to say, the along-strike transition from oceanic lithosphere to continental crustal material on the downgoing Austral-Indian plate, from the ^3He measurements, seems to be extremely sharp. Katili and

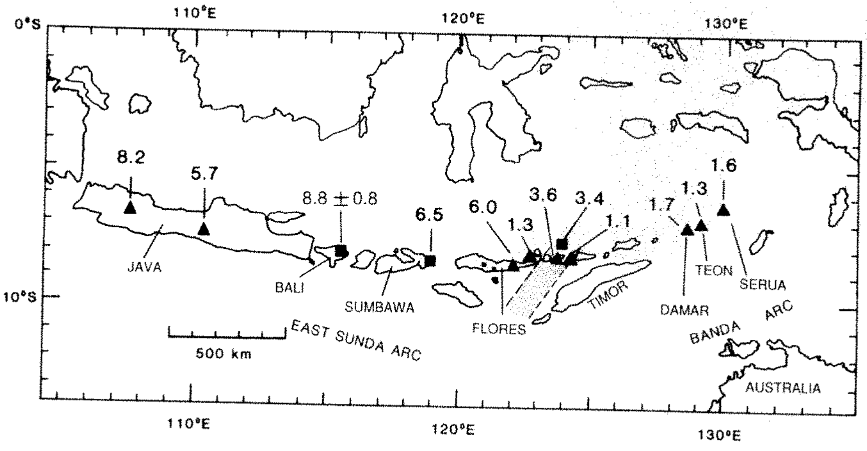

FIG. 1 Helium isotope ratios (relative to atmospheric helium) in volcanic gases (▲) and lavas (■) in the Indonesian volcanic arcs. From west to east in the Sunda arc the volcanoes are Tangkuban Parahu and Merapi on Java, Batur on Bali, Sangeang Api on Sumbawa, Keli Mutu and Egon on Flores, Lewotolo on Lomblen, Batu Tara (a potassium-rich leucitic volcano) in the Banda Sea 53 km north of Lewotolo, and Sirung on Pantar. Between the active Sunda and Banda volcanoes is a 500-km volcanoseismic 'quiet sector' extending from Pantar to Damar. Dashed lines correspond to proposed faults in the Savu Basin (stippled): the eastern fault runs east of Pantar; the western fault is assumed to run east of Flores[19] or west of Pantar[24,25]. Volcano names follow the usage of Simkin *et al.*[28].

TABLE 1 Composition of Indonesian volcanic gases

Location	Sample type*	T(°C)	R/R_A	$\frac{\text{He/Ne}}{(\text{He/Ne})_A}$	R_c/R_A	N_2/Ar	He/NC† (p.p.m.)
Java							
Tangkuban Parahu	as	57	8.16	991	8.17	856	450
Merapi Craters							
Gendol	sf	460	4.54	4.0	5.70		
Gendol	sf	460	4.50	4.7	5.43	85.0	27.3
Woro	sf	500	3.49	2.2	5.62	84.9	14.0
Woro	sf	500	3.40	2.1	5.49		
Bali							
Batur	ol				8.8 ± 0.8		[He] = 2.1×10^{-9} cm^3 g^{-1}
Sumbawa							
Sangeang Api	cpx				6.52		[He] = 6.6×10^{-9} cm^3 g^{-1}
Flores							
Keli Mutu	wa	51	5.92	68.6	6.00	48.9	[He] = 2.3×10^{-6} cm^3 g^{-1}
Egon I	sf	124	1.27	312	1.27	134	898
Egon II	sf	98	1.23	83.2	1.23	99.4	440
Lomblen							
Lewotolo							
Loc.1	sf	140	3.67	2,503	3.67	402	4,194
Loc. 3	sf	300	3.45	185	3.47	161	1,375
Loc. 5	sf	333	2.96	4.0	3.61		
No. 1‡	sf	490	3.62	500	3.62		3,094
No. 2‡	sf	490	3.57	1,1180	3.57		4,163
Batu Tara	cpx				3.44		[He] = 1.5×10^{-9} cm^3 g^{-1}
Pantar							
Sirung‡	sf	95	1.03	1.7	(1.07)		
Damar‡	ff	135	1.69	600	1.69	239	11,120
‡	sf	170	1.65	3,200	1.65	179	11,600
Teon‡	sf	99	1.31	130	1.31	98.5	1,719
Serua‡	sf	102	1.27	1.7	1.64		

We used a Clarke-type ^{3}He mass spectrometer (GAD) for the ^{3}He/^{4}He measurements[1,2]. Helium was separated from neon on activated charcoal at 37 K and measured relative to the Yellowstone 'Murdering Mudpots' standard ($R/R_A = 16.45$). Neon was also measured and the He/Ne ratios (column 5) were used to correct the measured ratios (column 4), assuming all Ne to be atmospheric. R_c is the corrected ratio and R_A is the ratio in atmospheric helium. Precision of the ^{3}He/^{4}He ratios is 1%. The helium was extracted from phenocrysts in recent lavas by vacuum crushing: [He] is the measured He concentration. Blank contribution to ^{3}He was 0.7% (Sangeang Api), 7% (Batur) and 10% (Batu Tara). Only 0.5 g of olivine was available for the Batur analysis: a 10% uncertainty is assigned to the ratio of this sample.

* Sample key: sf, summit fumarole; ff, flank fumarole; as, gas from acid spring; wa, hotspring water sample; ol, cpx, olivine and pyroxene phenocrysts in lavas. Lava samples: Batur, mixture of 1904 and 1974 flows; Sangeang Api, xenolith or cumulate sample 'B4'[13] (also R. Varne No. 48083) from 1911 lahar on south-west coast; Batu Tara, potassium-rich leucite basanite[9,16] (R. Varne No. 67130).

† NC, Non-condensable gases. Samples were collected in evacuated 1720-glass flasks (50-ml volume) with vacuum stopcocks. Analyses were made using thermal conductivity gas chromatography, after liquid nitrogen separation of CO_2, H_2S and SO_2 (data to be reported elsewhere). Hydrogen was observed only qualitatively in our system: significant amounts of H_2 were present in the Tangkuban Parahu and Lewotolo samples and traces were seen in the Merapi analyses. Methane was found in gases from Tangkuban Parahu (6.2% of NC), Lewotolo (2.4% of NC) and Damar ff (0.3% of NC).

‡ The ^{3}He and He/Ne data for these samples are from our previous work[2]. (He/NC ratios tabulated for these samples were calculated incorrectly). The ^{3}He/^{4}He ratio for Sirung is questionable because of the large amount of air helium in this sample.

Hartono[5] placed this transition zone "in the eastern part of Flores Island", in close agreement with the position located here by helium isotope ratios.

Within the transition zone the ^{3}He structure is marked by higher R/R_A values at Lewotolo and Batu Tara in the centre and more radiogenic ratios at the two volcanoes, Egon and Sirung, that define the edges. Although this pattern is not yet explained there are a number of possibilities. Wheller *et al.*[9] identified three sectors along the Sunda arc from Java to Lomblen that are terminated by the potassium-rich, leucite-bearing volcanoes noted above. These mark the ends of volcanic sectors with progressively increasing K/Si ratios, after which the series begins again in the next sector. Their 'Flores sector' is terminated by the potassium-rich volcano Batu Tara that, with Lewotolo—a high-potassium volcano—marks the centre of the ^{3}He transition zone (Fig. 2). Wheller *et al.* show that K, Si, and ^{87}Sr/^{86}Sr data for Sunda-Banda lavas require, in addition to ocean mantle and continental crust sources, a third, potassium-rich, component. This component may be the source of the elevated ^{3}He/^{4}He ratios in the central part of the ^{3}He transition zone.

An alternative explanation may lie in the relationship between volcanism and tectonics in the Savu Basin, a depression with large negative gravity anomalies which is floored by oceanic crust and contains the ^{3}He transition zone between the islands of Flores and Pantar. Nishimura *et al.*[20] showed that the transition zone is a discontinuity marked by offsets of gravity anomaly, earthquake patterns, submarine morphology, strike of the volcanic arc and chemistry of Quaternary lavas. McBride and Karig[21] interpreted the gravity data as evidence for the subduction of continental material into the mantle below the basin, and Eva *et al.*[22] have proposed that the strike of the subducting slab is parallel to Timor in the eastern part of the basin, resulting in transcurrent faulting such as the Pantar fracture zone deduced from seismic data[23]. Silver *et al.*[24] and van Bergen *et al.*[25] show cross-arc faults both east and west of Pantar in the eastern part of the transition zone, whereas Varekamp *et al.*[19] propose that the Savu Basin is bounded east and west by NNE to SSW transcurrent faults crossing the arc tangent to the east side of Pantar and the eastern end of Flores, almost exactly the arc section outlined by the ^{3}He transition zone. Such cross-arc fault patterns might provide zones for trapping subducted continental material at the boundaries of the Savu Basin while allowing less-contaminated mantle material to erupt in the centre of the basin

Perhaps the most difficult question posed by our data is the very low ^{3}He/^{4}He ratio (1.25 R_A) at Egon Volcano on eastern Flores. Hamilton (ref. 4, Figs 53 and 78) indicates that continen-

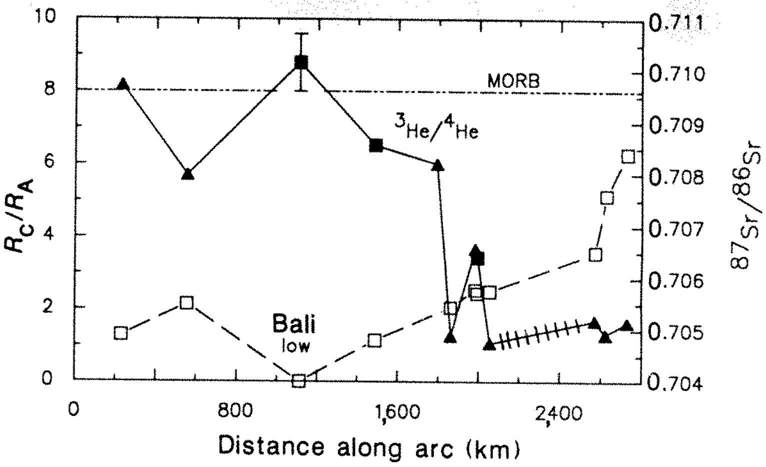

FIG. 2 ^{3}He/^{4}He and ^{87}Sr/^{86}Sr ratios along the Sunda–Banda arcs from the western end of Java. Symbols as in Fig. 1: geographic coordinates are from ref. 18. MORB, ^{3}He/^{4}He ratio ($R/R_A = 8 \pm 1$) in mid-ocean-ridge-basalt helium[2,27]. Sr isotope data (open squares) are from refs 8–16 (the dashed lines are profiles from refs 6, 10 and 19). The lowest (least radiogenic) ^{87}Sr/^{86}Sr ratios are in lavas from Bali: eastward from Bali the Sr ratios increase monotonically to the sharp increase in slope at the end of the Banda quiet zone (cross-hatched). The ^{3}He/^{4}He ratios, however, drop very sharply (factor of five) in the 68-km span from Keli Mutu to Egon. The antithetic correlation of Sr and He ratios in the Sunda arc is consistent with simple two-component mixing. Helium data from two sources, fumarole gases and lava phenocrysts, are compared with the Sr isotope ratios. The only detailed comparison so far of such sources is of data from the Mariana arc[2]: in this area helium from one clinopyroxene and two olivine samples from two seamounts and from a hotspring on Agrigan volcano[1,2] had identical ^{3}He/^{4}He ratios. But it was also found[2] that 'flank' fumaroles on large volcanoes such as Makushin tend to have lower ^{3}He/^{4}He ratios than 'summit' fumaroles: thus caution is necessary when flank fumaroles are compared with other sources.

tal crust is subducted to a depth of, at most, ~40 km below the Savu Basin north of western Timor, and so that only old (Jurassic) oceanic crust lies below Flores (and Pantar). The previous studies of arc helium[1,2] show, however, that subduction of oceanic crust does not result in low (radiogenic) ^{3}He/^{4}He ratios, so that unless the Egon samples have been contaminated with near-surface radiogenic helium, a source of continental material is required at depth. Hamilton (personal communication) suggests that subducting sediments from abyssal fans north-west of Australia, derived from Precambrian crust, could well be present below eastern Flores and the transition zone to supply the observed helium. But McCaffrey *et al.*[26], who carried out a detailed microearthquake study in the Savu Basin, found a sharp seismic boundary between oceanic and continental crust striking ~N 65° E between Timor and Pantar, which they interpreted as a bend in the subducting slab of continental crust, extending to a depth of 150 km. This would put continental material beneath Pantar and probably beneath Solor (ref. 4, Fig. 78) at the eastern end of Flores. Our data are not sufficient for us to choose between these interpretations of the geophysical results but, with more extensive sampling across the ^{3}He transition zone, it may be possible to track the subducted continental crust across this zone.

Figure 2 also shows the striking antithetic correlation between helium and strontium isotopes along the Sunda arc. A similar correlation has been observed in restricted regions of the Mid-Atlantic Ridge[27], but this is the first clear case for two-component mixing of helium and strontium over a major segment of an active plate boundary. The antithetic He–Sr relationship is not present in the Banda arc: ^{3}He/^{4}He ratios are almost constant whereas ^{87}Sr/^{86}Sr ratios continue to increase along the arc. The lowest ^{87}Sr/^{86}Sr ratio in in the Indonesian arcs is ~0.7040 in the lavas of Bali[10]: these define a 'Bali low' in Fig. 2, close to the value for the MORB-like endmember, and correspond to an increased ^{3}He/^{4}He ratio: a 'Bali high' relative to the rest of the arc. □

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1. Craig, H., Lupton, J. E. & Horibe, Y. in *Terrestrial Rare Gases* (eds Alexander, E. C. & Ozima, M.) 3–16 (Japan Sci. Soc., Tokyo, 1978).
2. Poreda, R. J. & Craig, H. *Nature* **338**, 473–478 (1989).
3. Katili, J. A. *Tectonophysics* **26**, 165–188 (1975).
4. Hamilton, W. *U.S. Geol. Surv. Prof. Pap.* **1078**, 1–345 (1979).
5. Katili, J. A. & Hartono, H. M. S. in *Geodynamics of the Western Pacific-Indonesian Region* (eds Hilde, H. C. & Uyeda, S.) 387–399 (Am. Geophys. Un., Washington, DC, 1983).
6. Jezek, P. A. & Hutchinson, C. S. *Bull. volcan.* **41**, 586–608 (1978).
7. Whitford, D. J., Nicholls, I. A. & Taylor, S. R. *Contr. Miner. Petrol.* **70**, 341–346 (1979).
8. Foden, J. D. & Varne, R. *Chem. Geol.* **30**, 201–206 (1980).
9. Wheller, G. E., Varne, R., Foden, J. D. & Abbott, M. J. *J. Volcan. geotherm. Res.* **32**, 137–160 (1987).
10. Whitford, D. J. *Geochim. cosmochim. Acta* **39**, 1287–1302 (1975).
11. Varne, R. & Foden, J. D. in *The Origin of Arcs* (ed. Wezel, F. C.) 159–189 (1986).
12. Whitford, D. J., Compston, W., Nichols, I. A. & Abbott, M. J. *Geology* **5**, 571–575 (1977).
13. Whitford, D. J., Foden, J. & Varne, R. *Carnegie Inst. Wash. Ybk.* **77**, 613–620 (1978).
14. Whitford, D. J. & Jezek, P. A. *Contr. Miner. Petrol.* **68**, 141–150 (1979).
15. Whitford, D. J., White, W. M. & Jezek, P. A. *Geochim. cosmochim. Acta* **45**, 989–995 (1981).
16. Stolz, A. J., Varne, R., Wheller, G. E., Foden, J. D. & Abbott, M. J. *Contr. Miner. Petrol.* **98**, 374–389 (1988).
17. Whitford, D. J. & Jezek, P. A. *Bull. Geol. Soc. Am.* **93**, 504–513 (1982).
18. Neumann van Padang, M. *Catalogue of the Active Volcanoes of the World, I: Indonesia* xiii (Int. Volc. Ass., Napoli, 1951).
19. Varekamp, J. C. *et al. Netherlands J. Sea Res.* (in the press).
20. Nishimura *et al. Bandung Geol. Res. Dev. Cent., Spec. Publ. No. 2*, 105–114 (1981).
21. McBride, J. H. & Karig, D. E. *Tectonophysics* **140**, 265–273 (1987).
22. Eva, C., Cattaneo, M. & Merlanti, F. *Tectonophysics* **146**, 241–259 (1988).
23. Nishimura, S. & Suparka, S. *J. Southeast Asian Earth Sci.* **1**, 45–57 (1986).
24. Silver, E. A. *et al. Geology* **13**, 687–691 (1985).
25. Van Bergen *et al. Netherlands J. Sea Res.* (in the press).
26. McCaffrey, R., Molnar, P., Roecker, S. W. & Joyodiwiryo, Y. S. *J. geophys. Res.* **90**, 4511–4528 (1985).
27. Kurz, M. D., Jenkins, W. J., Schilling, J.-G. & Hart, S. R. *Earth planet. Sci. Lett.* **58**, 1–14 (1982).
28. Simkin, T. *et al. Volcanoes of the World* (Hutchinson Ross, Stroudsburg, 1981).

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Leakage of helium from the Pannonian basin

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THE aquifer system of the Great Hungarian Plain covers 50,000 km^2 and contains helium with an isotopic composition that indicates regional loss of both radiogenic-crustal and mantle-derived helium. Mantle-derived melts must be present at depth over much of the region. Here we show that the mantle helium flux through the basin may be supported by the addition of 20 m of melt per Myr. Considerations of hydrogeology and helium abundances show that the rate of loss of radiogenic helium from the basin may exceed the crustal helium production rate.

All continental ground waters contain small amounts of dissolved helium, often considerably in excess of the equilibrium saturation value. Most of this helium is generated by radioactivity and other nuclear processes within the crust. Such helium is characterized by its isotopic composition,

$R = {}^3\text{He}/{}^4\text{He} \sim 10^{-8}$. In some areas, however, volatiles escape from the mantle or lower crust and accumulate in the groundwater system. The presence of another helium component derived from the mantle may be established by its distinctive isotopic composition ($R \simeq 10^{-5}$).

In principle the fluxes of both mantle-derived helium and radiogenic crustal helium may be estimated if the characteristics of the aquifer are known. An ideal case would be a regional groundwater system of known area and well defined limits, for which the recharge and discharge were known and in balance. For such a result to be regionally significant, the extent of the aquifer system should be large by comparison with the thickness of the crust and direct losses of gas from the crust to the atmosphere should be small. In many respects the Great Hungarian Plain (GHP) within the Pannonian Basin meets these requirements, although there is no way of knowing how much gas is lost directly to the atmosphere and what proportion is trapped by the ground water.

The Pannonian basin in eastern Central Europe is an oval sedimentary basin, with a diameter of ~500 km (Fig. 1*a*). The sedimentary fill comprises early Miocene, Pliocene and Quaternary deposits that are predominantly clastic, often with significant amounts of rhyolite tuffs and lava flows[1]. The basin is bounded by the Alps, Carpathians and Dinarides, and constitutes a closed hydrological system[2,3]. The GHP forms the eastern part of the Pannonian basin and is a remarkably flat area between 80 and 200 m above sea level.

The Quaternary consists of poorly consolidated sand and clayey sand deposits (100–600 m) overlying 500–1,800 m of Pliocene lacustrine and fluviatile alternating sands and clays. The underlying Miocene is a thick (up to 4,000 m) shale-rich sequence overlying a basement that varies in age from late Palaeozoic to Mesozoic and, locally, to Oligocene. The structural development of the GHP has been controlled by a set of transcurrent faults striking east-north-east to west-south-west, and associated normal faults[4,5].

There are two water flow regimes of regional extent in the GHP[2,6] (Fig. 2). The upper flow regime is essentially within the Quaternary. Meteoric water recharge occurs at topographic highs (Figs 1*b* and 2) and discharge occurs in topographic lows, particularly into the base of the Tisza River. A lower, dominantly connate, saline-water flow regime (Fig. 2) exists within the Pliocene and part of the Miocene. The Pannonian Basin is artesian, with the upward flow controlled by overpressuring of deeper waters. Some communication between the two flow regimes occurs in the central part of the GHP (Fig. 2).

Hydrological observations indicate[5-8] that the total groundwater flow in the GHP is $\sim 35\ \text{m}^3\ \text{s}^{-1}$. This value is based on some thousand pressure observations and permeability estimates from pumping experiments in boreholes. This is consistent with a previous estimate[9] of $17 \pm 11\ \text{m}^3\ \text{s}^{-1}$ for the discharge of subsurface water into the Tisza River system, which provides a lower limit for the discharge of the basin system as a whole. This latter estimate was derived from the progressive dilution of tritiated Tisza river water by non-tritiated sub-surface water over a distance of some 300 km.

The locations of samples analysed either in Cambridge or in Heidelberg are shown in Fig. 1*b* and the results summarized in Table 1. We collected the samples in conventional clamped annealed copper tubes and analysed them using very similar techniques. The methods used in Cambridge have been described previously[10]. We estimate associated experimental uncertainties to be ≤10% for helium abundances and ≤3% for isotope ratios.

Comparison of Table 1 with Fig. 1*b* shows that all samples contain more helium than the normal air saturation value ($4.64 \times 10^{-8}\ \text{cm}^3\ \text{g}^{-1}$ at 10 °C) and that the excess increases with distance from the recharge area and reaches a maximum two to three orders of magnitude higher than the saturation value. For some of the Pliocene waters we consider the measured value to be only a lower limit as their very low neon contents indicate that they have undergone degassing at some stage.

We obtain a crude estimate of the helium content of the discharging waters by averaging the values measured in Quaternary waters of the discharge zone. As far as we know, the Pliocene waters do not discharge directly to the surface but first mix with the overlying Quaternary waters.

The samples with the highest concentrations come from the central part of the discharge zone and may be the most representative of the helium contents of waters on their return to the surface. If so the simple average, $6.3 \times 10^{-6}\ \text{cm}^3\ \text{g}^{-1}$, is a lower limiting value. This represents the helium that has been flushed from the rocks through which the waters have passed on their way from recharge to discharge. The R/R_A values where R_A is the ratio for the atmospheric standard given in Table 1 show that although most of the helium is radiogenic

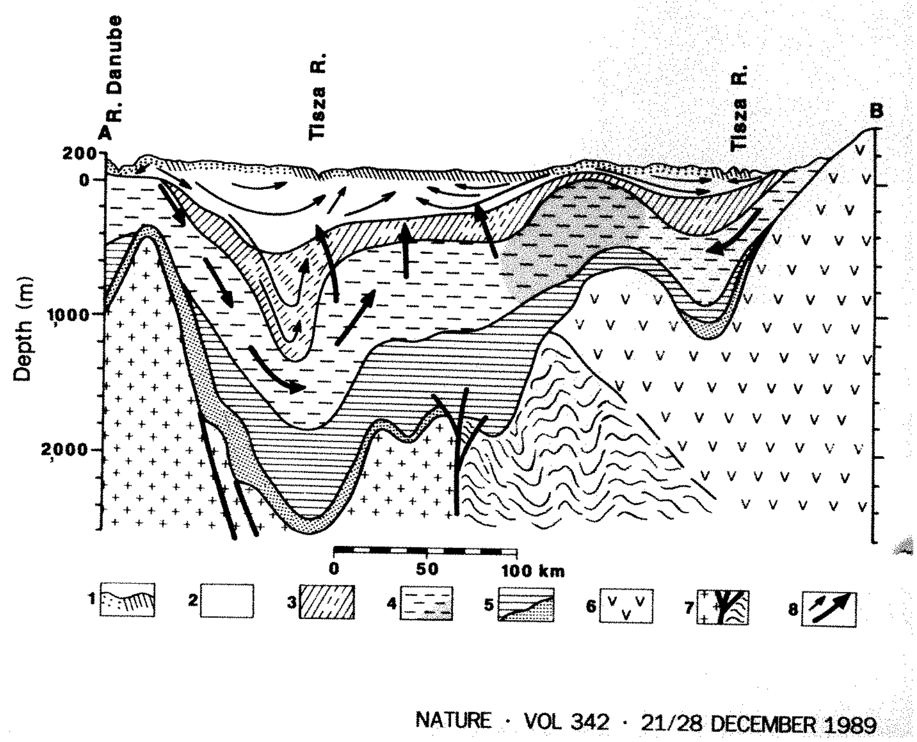

FIG. 2 Generalized cross-section to show the water flow regimes in the sedimentary rocks of the Great Hungarian Plain. The section crosses in south-west/north-east direction the area of Fig. 1*b*. Key: 1, permeable and impermeable deposits at the surface; 2, highly permeable Quaternary beds; 3, Upper Pliocene consisting of impervious and permeable layers; 4, Pliocene sequence of permeable rocks containing fossil sea water and fresh water; 5, late and middle Miocene, mostly aquiclude; 6, late and middle Miocene volcanic rocks; 7, basement of the sedimentary basin consisting of Mesozoic or older rocks and their tectonic contact with Palaeogene flysch; 8, water flow direction in the upper aquifer (thin arrow) and lower aquifer (thick arrow).

TABLE 1 Isotope data for Quaternary and Pliocene aquifers

Sample locality		Depth (metres)	Helium† (10^{-8} cm^3 g^{-1})	Neon‡ (10^{-7} cm^3 g^{-1})	R/R_A§
Samples from Quaternary aquifers					
a Recharge zone					
1	Kölcse*	90–116	11.1	—	—
2	Mánd*	73–120	32.6	—	—
3a	Fehérgyarmat	82–96	25.6 (0.93)	—	0.33‖
3b	Fehérgyarmat*	165–189	25.1	—	—
4	Mátészalka*	226–251	9.2	—	—
5a	Nyírbátor	161–193	5.5	—	—
5b	Nyírbátor*	297–313	11.4	—	—
6	Nyírlugos*	110–175	4.9	—	—
7	Nyíradony*	244–254	5.7	—	—
8a	Debrecen	25–49	4.9	—	—
8b	Debrecen	144–169	5.0	—	—
8c	Debrecen	70–92	6.8	—	—
8d	Debrecen*	138–181	21.3	—	—
b Discharge zone					
9	Hajdúszoboszló*	<118	1,390 (117)	—	0.71
10	Kaba*	182–209	1,030	—	—
11	Püspökladány*	192–215	1,040	—	—
12	Kunmadaras	260–294	512 (34.7)	1.00	0.58
14b	Kisujszállás	56–72	1,030	—	—
14c	Kisujszállás	93–106	900	—	—
14d	Kisujszállás	239–257	1,270 (126)	—	0.83
14e	Kisujszállás	509–598	730	—	—
15	Örményes	258–286	408 (19.5)	—	0.42
17	Mezőtúr	92–122	223 (21.1)	3.87	0.47
18	Mezőtúr	161–170	309 (25.2)	1.45	0.69
19	Kétpo	390–405	782 (42.2)	1.82	0.47
20	Törökszentmiklós	239–281	208 (4.7)	2.59	0.22
21	Tiszakécske	211–220	182 (27.7)	1.27	1.25
22	Lakitelek	—	307 (52.8)	1.28	1.41
34a	Tiszacsege	198–210	292 (9.9)	—	0.31
45	Kunhegyes	288–344	425	—	—
46	Balmazujvaros	85–98	286	—	—
Samples from Pliocene aquifers					
a Recharge zone					
23	Abasár*	11–19	5.3	—	—
24	Gyönyös*	140–194	7.2	—	—
25	Gyöngyös*	191–239	6.0	—	—
26	Karácsond*	274–330	193	—	—
27	Nagyfüged*	282–332	19.0	—	0.39‖
28	Tarnaméra*	435–594	98.6	—	—
29	Boconád*	446–467	46.6	—	0.35‖
30	Heves*	360–387	27.1	—	0.67‖
31	Heves*	674–772	169	—	0.23‖
32	Hevesvezekény*	541–568	40.6	—	—
b Discharge zone					
34b	Tiszacsege	1,085–1,271	484¶	0.44¶	0.32
35	Tiszafüred	719–970	229¶	0.91¶	0.11
36	Tiszaörs	716–1777	53.0¶	0.086¶	0.44
37	Hajdúböszörmény	598–636	8900	3.81	0.22
38	Szolnok	1,017–1,125	14.5¶	0.024¶	0.38
39	Hajdúszoboszló	381–434	277¶	0.17¶	0.72
40	Hajdúszoboszló	533–684	1,030	1.87	0.71
41	Karcag	1,190–1,334	128¶	0.06¶	0.25
42a	Mezötúr	602–806	—	—	0.56
42b	Mezötúr	924–1034	779	3.87	0.47
43	Törökszentmiklós	995–1127	1,510¶	0.28¶	0.83
44	Tiszakécske	925–1003	76	3.42	1.39
8e	Debrecen	826–966	1,510	58.2	0.11

* Data from ref. 12.

† Total helium content (mantle-derived component in parentheses), assigned uncertainty ±10%.

‡ Total neon content, assigned uncertainty ±20%.

§ Measured $^3He/^4He$ ratio relative to an atmospheric standard.

($R_A=1.4\times10^{-6}$), corrected for atmospheric component using measured neon concentration following the method in ref. 21.

Values uncorrected for atmospheric component because of lack of neon concentration data.

ow neon concentration indicates some helium depletion.

and probably of crustal origin a small but variable amount must in every case be derived from the mantle. Assuming a value of $R/R_A = 8.0$ for mantle helium the proportion varies from 1 to 16%.

We consider the origin and significance of these components separately. All ground waters from the Pannonian Basin analysed for helium isotopes so far[11,12] (Table 1) have R/R_A values exceeding the radiogenic production ratio for crustal helium. This is also true for over one hundred other analyses we have made elsewhere in Hungary[13]. We consider the excess ^{3}He to be mantle derived and to be pervasively invading the crust, presumably along with other volatiles, over much of the Pannonian Basin. We obtain a total flow of 15 $cm^3 s^{-1}$ (STP) for mantle-derived helium through the GHP from the product of the average mantle helium content of Quaternary discharge waters (Table 1) and the water flow rate (35 $m^3 s^{-1}$). Over the 5×10^4-km^2 area of the GHP the 15 $cm^3 s^{-1}$ mantle helium flow rate corresponds to $\sim 8 \times 10^4$ atom ^{3}He $m^{-2} s^{-1}$. This estimate is necessarily crude, because of the uncertainty in the average mantle helium content of the aquifer, but for the reasons given earlier it probably represents a lower limit and it does provide a useful comparison with the globally averaged mantle ^{3}He flux from the ocean ridges of 4×10^4 atom $m^{-2} s^{-1}$. This is several orders of magnitude higher than in stable, atectonic continental areas[14,15].

Mantle helium has been shown to escape through the crust over much of southern and western Europe[14,16,17]. It was argued that the most plausible means of maintaining such a flux of mantle volatiles was by the emplacement of small volumes of mantle melt in the lower crust, but over a much wider area than the surface volcanic manifestations indicate. Surface Plio-Pleistocene basaltic volcanics are present only sporadically in Hungary such as in the Little Hungarian Plain to the west of the area studied here, but have also been recorded from a borehole in the Great Hungarian Plain[18].

The amount of melt addition beneath the Pannonian Basin is uncertain and could be very small. Because helium behaves as a highly incompatible element and fractionates into the first few per cent of partial melt, the total amount of helium extracted by melting does not depend greatly on the size of the melt fraction. For example, at ocean ridges $\sim 4 \times 10^{-3}$ mol of helium ($R/R_A \sim 8$) are released from the mantle for every cubic metre of basaltic ocean crust formed. If the mantle helium leaking from the Pannonian Basin is supported by basalt with a similar concentration of helium this would require[14] the addition to the crust of $\sim$110 m of basalt per million years. In a sub-continental environment, however, large melt fractions (for example, 10%) such as those at spreading ridges are unlikely and for a 2% melt fraction, which is more likely given the alkalic nature of Plio-Pleistocene basalts in the Pannonian Basin[18], the rate of addition would be 20 m Myr^{-1}.

The greater part (>90%) of the helium contained in the aquifer system is radiogenic and has been derived from the underlying continental crust, where its generation is associated with the production of radiogenic heat with the approximate relationship 10^{12} atom ^{4}He J^{-1} (ref. 19). The production of heat within the crust of the GHP is estimated to be 25 mW m^{-2} (±50%), which is within the range for average continental crust and corresponds to an equivalent flux of 2.5×10^{10} atom ^{4}He $m^{-2} s^{-1}$. We compare this with the value of $\sim 8 \times 10^{10}$ atom ^{4}He $m^{-2} s^{-1}$ derived by dividing the total crustal helium flux by the area of the basin. Given the uncertainties associated with both values we do not regard them to be significantly different and, considering the persistence of relatively high sub-surface temperatures in Hungary[20] since the late Tertiary, it would be surprising if surface helium loss departed very greatly from the production rate. If further work were to substantiate the difference, however, it would be necessary to consider possible addition of stored 'fossil' ^{4}He from the deeper aquifer systems.

We now consider the broader relationship between heat and helium in the Pannonian Basin, particularly in the light of the studies made on high-temperature hydrothermal vents on mid-ocean ridges[15]. No systematic relationship was found between water temperature and either isotopic composition or concentration of helium and it would perhaps be suprising if such a relationship existed. The aquifer system is known to have an important effect of surface heat flow in the Pannonian Basin[2,20] but primarily by redistributing heat in the upper one or two kilometres of the crust, depressing temperatures in down-flow areas and elevating them where flows ascend. The major contributions to surface heat flow, including crustal radiogenic and sub-crustal sources, originate below the circulation depth. The circulations seem to be principally driven by hydrological head with only minor perturbations by convective thermal instabilities. By contrast, quite different factors control the helium inventory and its isotopic composition: in the case of the crustally derived radiogenic component, it is the age of the waters and to some extent variations in crustal chemistry; in the case of the mantle component, it is probably the distribution of faults and joints that localize the ascent of volatiles from below.

The rates of magmatic addition to the crust discussed earlier are sufficiently low that if they were more or less uniform across the basin, they would make an addition to the crustal heat flow that was undetectable against the major contributions from crustal radioactivity and conduction through the base of the lithosphere; together the latter sustain an average surface heat flow of $\sim$80 mW m^{-2} in the Pannonian Basin[19] whereas magmatic addition at a rate of 20 m Myr^{-1} would contribute only 2 mW m^{-2}. It also follows that the processes transporting helium released by the magma through the crust are unlikely to make a significant contribution to the transport of heat; although hard to substantiate, it is likely that the helium is transported by carbon-dioxide-rich fluids that are of insufficient mass to affect the temperature in the regions through which they pass. □

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1. Horváth, F. & Berckhemer, H. *Alpine-Mediterranean Geodynamics* (eds Berckhemer, H. & Hsu, K.) *Am. Geophys. Un. & Geol. Soc. Am. Publs, Geodyn. Ser. 7* 141–173 (1982).
2. Erdélyi, M. *J. Geodyn.* **4,** 321–330 (1985).
3. Böcker, T. & Liebe, P. *Exploitation Possibilities of Karstic Stratum Water* (in Hungarian.) Internal Rep. (VITUKI, Budapest, 1980).
4. Rumpler, J. & Horváth, F. *The Pannonian Basin: a Study in Basin Evolution, Am. Ass. Petrol. Geol. Mem.* No. 45. 153–170 (1988).
5. Royden, L., Horváth, F. & Rumpler, J. *Tectonics* **2,** 63–90 (1983).
6. Stegena, L. in *Hydrogeological Regimes and their Subsurface Thermal Effects, Am. geophys. Un, Monogr.* **47,** 81–86 (1989).
7. Toth, J. *Am. Ass. Petrol. Geol., Studies in Geology* Vol. 10, 121–167 (1980).
8. Alföldi, L., Galfi, J. & Liebe, P. *J. Geodyn.* **41,** 199–218 (1985).
9. Deák, J. *Isotope Techniques in Groundwater Hydrology* Vol. 1, 157–167 (Int. Atom. Environ. Ag., Vienna, 1974).
10. Hooker, P. J., Bertrami, R., Lombardi, S., O'Nions, R. K. & Oxburgh, E. R. *Geochim. cosmochim. Acta* **49,** 2505–2513 (1985).
11. Deák, J., Stute, M., Rudolph, J. & Sonntag, C. *Proc. Int. Symp. on Use of Isotope Techniques in Water Resource Development* 335–350 (Int. Atom. Environ. Ag., Vienna, 1987).
12. Deák, J. *et al. The Pannonian Basin: a Study in Basin Evolution, Am. Ass. Petrol. Geol. Mem.* No. 45, 293–296 (1988).
13. Torgersen, T. & Clarke, W. B. *Earth planet. Sci. Lett.* **84,** 345–355 (1987).
14. O'Nions, R. K. & Oxburgh, E. R. *Earth planet. Sci. Lett.* **90,** 331–347 (1988).
15. Jenkins, W. J., Edmond, J. M. & Corliss, J. B. *Nature* **272,** 156–158 (1978).
16. Oxburgh, E. R. & O'Nions, R. K. *Science* **237,** 1583–1588 (1987).
17. Oxburgh, E. R., O'Nions, R. K. & Hill, R. I. *Nature* **324,** 632–635 (1986).
18. Balla, Z. *Earth Evolution Sci.* **1,** 240–247 (1981).
19. O'Nions, R. K. & Oxburgh, E. R. *Nature* **3,** 429–431 (1983).
20. Dövenyi, P., Horváth, F., Liebe, P., Galfi, J. & Erki, I. *Geophys. Trans.* **29,** 3–114 (1983).
21. Craig, H., Lupton, J. E. & Horibe, Y. in *Terrestrial Rare Gases* (eds Alexander, E. C. & Ozima, M.) 3–16 (Japan Scientific Society, Tokyo, 1978).

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dependent fractionation (ref. 16; M.R.P. and D. London, unpublished results). Little or no isotope fractionation takes place during hydrothermal leaching of boron from solid reservoirs[18,19].

Tourmaline-rich rocks are present in the Thackaringa, Broken Hill and Sundown Groups, but are most abundant in the upper part of the Broken Hill Group[20,21]. Concentrations of tourmaline are known in pelitic and psammitic metasedimentary rocks, in stratabound and stratiform tourmalinites, in lead-zinc-silver lodes, in stratabound tungsten deposits, in metamorphic segregations and veins, and in granitic pegmatites. Tourmaline separates from these diverse occurrences in the Broken Hill block all have very low $\delta^{11}\mathrm{B}$ values of −23.1 to −17.2‰ (Fig. 3). These values are unique relative to tourmalines from all other massive sulphide deposits and tourmalinites analysed to date[16], except for one tourmalinite from the Archaean Isua Group in West Greenland ($\delta^{11}\mathrm{B} = -22$‰, ref. 22).

We propose that the low $\delta^{11}\mathrm{B}$ values for the Broken Hill tourmalines reflect derivation of boron from source(s) containing isotopically light boron. The only natural reservoirs (Fig. 3) known to have sufficiently low $\delta^{11}\mathrm{B}$ values are borate minerals within non-marine evaporite sequences[23]. Geological data from the Broken Hill block are consistent with this interpretation, including the occurrence of stratiform albitites[24] in the Himalaya Formation (Fig. 2) which are considered to be the metamorphosed equivalents of analcime-rich tuffs or sediments altered in an alkaline sodic environment[3,25] and the presence of the Ettlewood Calc-Silicate Member at the base of the Broken Hill Group which is interpreted as a metamorphosed dolomite-chert unit[3,26]. Further evidence for evaporites includes the occurrence of scapolite locally in the Redan Gneiss and Ettlewood Calc-Silicate Member[3]. Abundant magnetite disseminated in quartzofeldspathic rocks of the Thackaringa Group[24] suggests protoliths of oxidized red beds; local magnetite-sillimanite rocks imply the former existence of bauxites or laterites[3]. Also, N. Herriman (personal communication) has recognized apparent crystal casts of the evaporite minerals kainite and gypsum in relatively undeformed rocks from the Black Prince mine (Fig. 1), which locally contain well preserved sedimentary structures[14,27]; the crystal casts may represent relict diagenetic minerals formed from a hypersaline interstitial brine.

Alternative interpretations of the Broken Hill tourmaline data include: (1) derivation of boron from low-$\delta^{11}\mathrm{B}$ Proterozoic sea water; (2) increased isotope fractionation during low-temperature deposition or diagenesis; (3) metamorphic fractionation. The first possibility is unlikely because tourmalines from other massive sulphide deposits of similar (1,750 ± 50 Myr) age, such as those in the Baltic Shield of Finland and Sweden, hav much higher $\delta^{11}\mathrm{B}$ values[16] (−12.3 to −2.0‰). Because hydrothermal fluids readily leach boron from sediments, and becaus most sediments have high concentrations of boron relative to sea water, the $\delta^{11}\mathrm{B}$ value of the fluid—and thus of precipitated tourmalines—is dominated by the $\delta^{11}\mathrm{B}$ value of the sediment

Low-temperature (<200 °C) formation of tourmaline could produce greater isotope fractionation between fluids and tourmaline and yield low $\delta^{11}\mathrm{B}$ values[16]. This possibility canno be completely excluded for Broken Hill, but there are several arguments against it. First, other stratabound lead-zinc deposits presumed to have formed at low temperatures such as those at Montauban (Quebec) and Balmat (New York) have associated tourmalines with much higher $\delta^{11}\mathrm{B}$ values of +1.6 and +6.7‰, respectively; tourmaline from the geologically similar Broken Hill lead-zinc-silver deposit in the Cape Province of South Africa is also isotopically heavier[16] (−13.8‰). Second, no relationship exists between the $\delta^{11}\mathrm{B}$ values of the Broken Hill tourmalines and their mineralogical, lithological or stratigraphic settings that might reflect different formation temperatures. Tourmalinites in the Globe mine section intimately associated with stratiform lead-zinc-silver lodes and manganese-garnet quartzites contain tourmaline with nearly identical $\delta^{11}\mathrm{B}$ values (−22.5 to −20.9‰) to those from tourmalinites associated with amphibolite and stratabound scheelite mineralization in the Corruga and Yanco Glen areas (−22.8 to −21.9‰). These data from the Broken Hill Group are similar to those for the Thackaringa Group, such as at Stirling Vale where tourmalinites occur locally in bedded albitites ($\delta^{11}\mathrm{B} = -19.7$‰). The similarity in $\delta^{11}\mathrm{B}$ values of tourmalines from these very different environments (and in others throughout the Broken Hill block) is evidence for a common source of the boron or a common process by which they acquired their distinctive $\delta^{11}\mathrm{B}$ values.

Metamorphic recrystallization of tourmaline could produce low $\delta^{11}\mathrm{B}$ values by preferential fractionation of the heavy boron isotope, $^{11}\mathrm{B}$, into a fluid phase or into other aluminosilicate minerals. There is, however, no correlation between tourmaline $\delta^{11}\mathrm{B}$ values and metamorphic grade in the region or with variations in mineral textures or mineral assemblages. Data for coarse-grained (>1 cm), metamorphically recrystallized tourmalines from the Silver King West mine ($\delta^{11}\mathrm{B} = -21.3$‰) and the Jersey Lily mine (−17.2‰) span a similar range of isotopic composition to that determined for fine-grained (<1 mm) tourmalines which apparently are not recrystallized. The relatively high value for the Jersey Lily sample may represent uptake of isotopically heavy boron released from the recrystalliz-

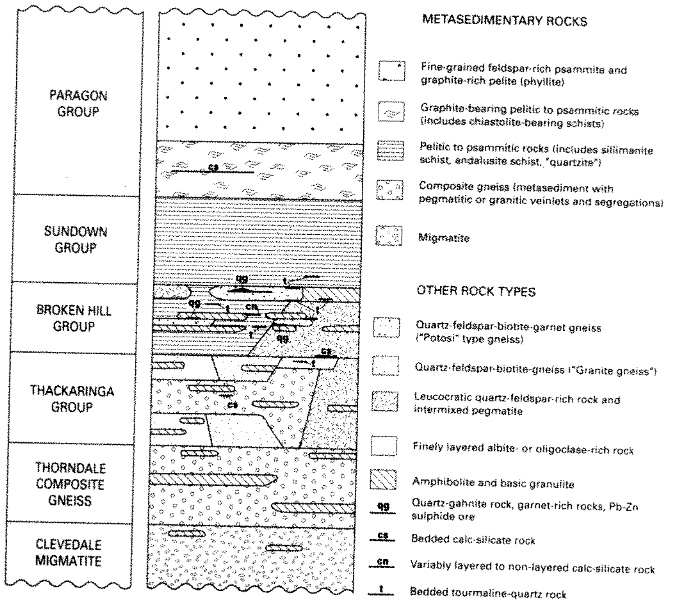
PARAGON GROUP
SUNDOWN GROUP
BROKEN HILL GROUP
THACKARINGA GROUP
THORNDALE COMPOSITE GNEISS
CLEVEDALE MIGMATITE
cs
qg
t
cn
METASEDIMENTARY ROCKS
Fine-grained feldspar-rich psammite and graphite-rich pelite (phyllite)
Graphite-bearing pelitic to psammitic rocks (includes chiastolite-bearing schists)
Pelitic to psammitic rocks (includes sillimanite schist, andalusite schist, "quartzite")
Composite gneiss (metasediment with pegmatitic or granitic veinlets and segregations)
Migmatite
OTHER ROCK TYPES
Quartz-feldspar-biotite-garnet gneiss ("Potosi" type gneiss)
Quartz-feldspar-biotite-gneiss ("Granite gneiss")
Leucocratic quartz-feldspar-rich rock and intermixed pegmatite
Finely layered albite- or oligoclase-rich rock
Amphibolite and basic granulite
qg Quartz-gahnite rock, garnet-rich rocks, Pb-Zn sulphide ore
cs Bedded calc-silicate rock
cn Variably layered to non-layered calc-silicate rock
t Bedded tourmaline-quartz rock

FIG. 2 Generalized lithostratigraphic sequence of the Broken Hill block after Willis *et al.*[2] and Barnes[9]. Total thickness of the sequence is ~7–9 km (refs 2, 3). Stratigraphic locations of important tourmalinites are schematically shown ('t'), based on work of Barnes[8,9,27], Willis *et al.*[2], and Stevens and Slack (unpublished results). Minor occurrences of tourmaline-rich veins, metamorphic segregations and pegmatites are not shown. The Himalaya Formation comprises upper part of the Thackaringa Group.

Boron isotope evidence for the involvement of non-marine evaporites in the origin of the Broken Hill ore deposits

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IDENTIFYING the palaeogeographic setting and mode of origin of stratabound ore deposits can be difficult in high-grade metamorphic terranes, where the effects of metamorphism may obscure the nature of the protoliths. Here we report boron isotope data for tourmalines from the early Proterozoic Broken Hill block, in Australia, which hosts giant lead–zinc–silver sulphide deposits. With one exception the $^{11}B/^{10}B$ ratios are lower than those for all other tourmalines from massive sulphide deposits and tourmalinites elsewhere in the world. We propose that these low ratios reflect leaching of boron from non-marine evaporitic borates by convecting hydrothermal fluids associated with early Proterozoic continental rifting. A possible modern analogue is the Salton Sea geothermal field in California.

The Broken Hill block is an inlier of early Proterozoic metamorphic and igneous rocks in western New South Wales, Australia (Fig. 1). Proterozoic rocks there consist of metasedimentary schists and gneisses and minor metavolcanic rocks[1-3] that have undergone complex post-depositional deformation and regional metamorphism[4-6], including granulite-facies pressure and temperature conditions developed in the southern part of the block[5]. The palaeotectonic setting of the region is generally considered to represent an intracontinental rift[2,3].

Detailed mapping studies[1-3] have recognized a coherent stratigraphy in the Broken Hill block (Fig. 2). The lowest stratigraphic units (Thackaringa Group and below) have recently been re-interpreted[3,7] as fluvio-deltaic and lacustrine (or coastal sabkha) sequences, overlain by shallow marine sands and shales comprising the Broken Hill, Sundown and Paragon Groups; tholeiitic basalt flows, tuffs or sills (presently amphibolites) and possible rhydodacitic volcanics (now quartzo-feldspathic gneisses) are mainly within the Thackaringa and Broken Hill Groups. A variety of stratabound and stratiform mineralization occurs in these same two groups[8,9] including the giant lead-zinc-silver lodes[10,11] stratigraphically within the upper part of the Broken Hill Group.

The borosilicate tourmaline is an accessory gangue mineral in many massive sulphide deposits, particularly those in dominantly clastic metasedimentary terranes[12,13]. Tourmaline is also the diagnostic mineral in stratabound tourmaline-rich rocks or tourmalinites, which are associated with a variety of stratabound (and commonly stratiform) mineral deposits[12,14,15]. Like massive sulphide deposits, most tourmalinites are believed to form by syngenetic or diagenetic processes before regional metamorphism[12-15]. Palmer and Slack[16] recently determined the boron isotopic composition of tourmaline in over 40 stratabound massive sulphide deposits and tourmalinites from diverse geological and tectonic settings worldwide. Figure 3 shows the range of $\delta^{11}B$ values obtained for tourmaline separates from these stratabound deposits ($n = 52$) and for Broken Hill ($n = 34$), together with the ranges observed for various fluid and solid boron reservoirs (the analytical details are given in ref. 16). The major control over the $\delta^{11}B$ values of the tourmalines is the composition of the boron source, especially the nature of footwall lithologies; secondary controls are water/rock ratios (indicated by $\delta^{18}O$-$\delta^{11}B$ relationships) and seawater entrainment (indicated by local geological settings)[16]. Tourmalines from dominantly clastic metasedimentary and metavolcanic terranes have intermediate $\delta^{11}B$ values (−15.7 to −1.5‰), whereas tourmalines from marine metaevaporite and carbonate-associated terranes have higher $\delta^{11}B$ values (typically >0‰). In both groups, tourmaline $\delta^{11}B$ values are systematically lower than that of the fluid from which they precipitated and that of the solid reservoirs from which the boron was originally derived. Boron isotope systematics are such that the light isotope, ^{10}B, is preferentially partitioned into the solid phase during fluid-solid interactions[16,17]. Under the pressure and temperature conditions inferred for massive sulphide and tourmalinite formation, tourmalines are interpreted to be 5-12‰ lighter than the boron-bearing fluid phase, mainly as a function of temperature-

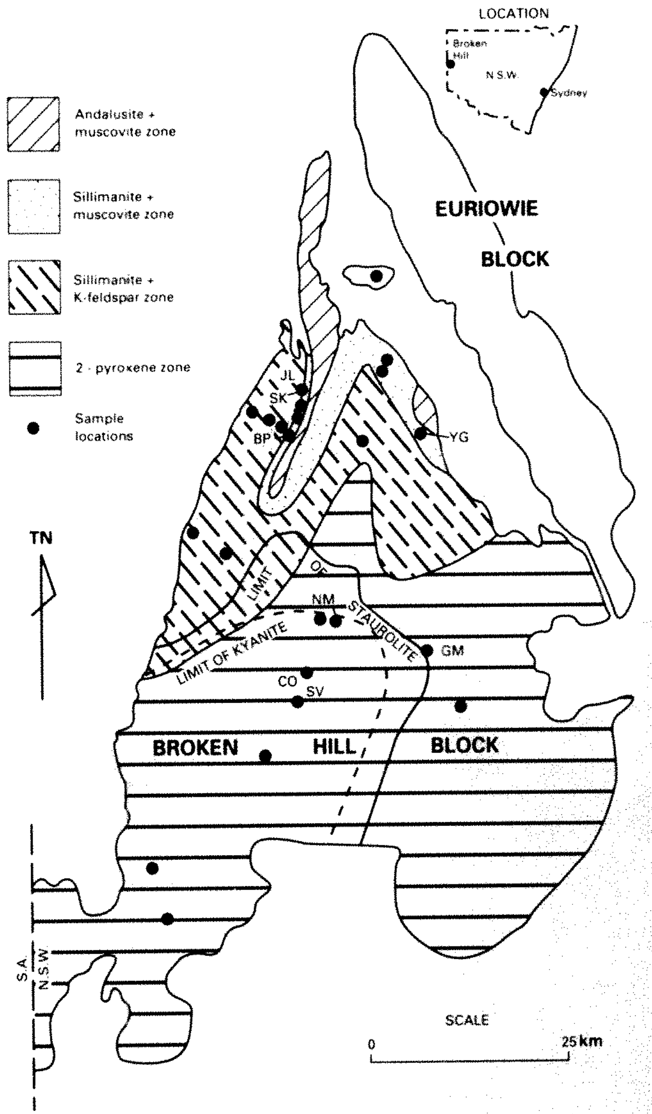

FIG. 1 Outline of the Broken Hill block showing metamorphic zones and sample locations. Prograde zones after Phillips[5]; retrograde zones (kyanite, staurolite) after Stevens[6]. We collected multiple samples at some localities. BP, Black Prince mine; CO, Corruga prospects; GM, Globe mine; JL, Jersey Lily mine; NM, Nine Mile mine; SK, Silver King West mine; SV, Stirling Vale homestead; YG, Yanco Glen area.

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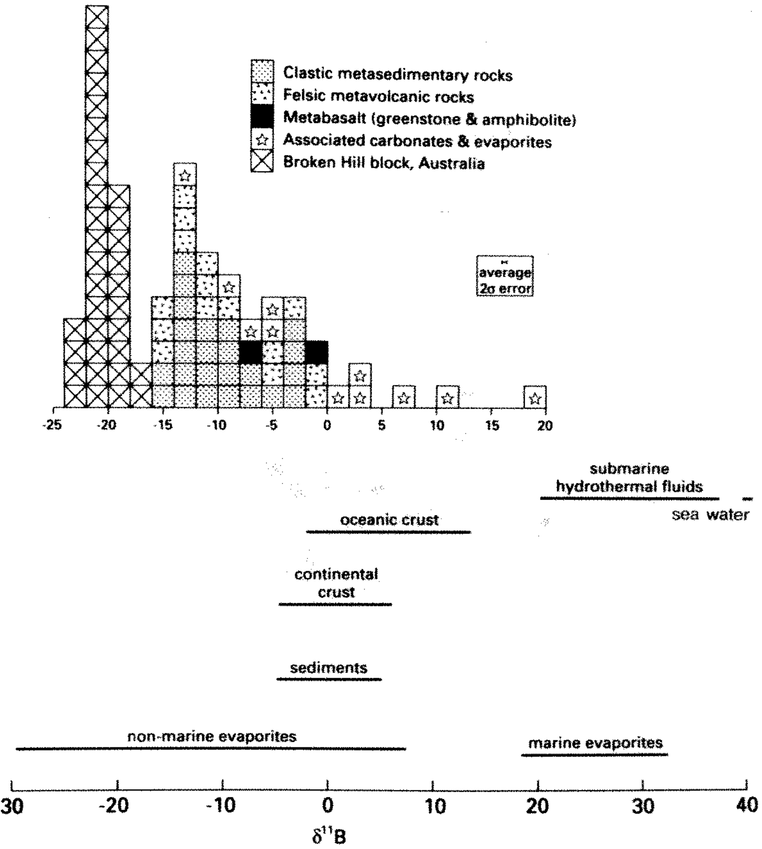

FIG. 3 Boron isotopic compositions of tourmaline (above) from the Broken Hill block compared with those from massive sulphide deposits and tourmalinites in geological settings elsewhere[16]. Lower scale shows the range of boron isotopic compositions identified for various fluid and solid boron reservoirs in nature[18,19,23]. $\delta^{11}B = [(^{11}B/^{10}B)_{sample}/(^{11}B/^{10}B)_{standard}] - 1$, where the standard is NBS boric acid SRM 951.

ation of local boron-bearing silicates (such as sillimanite[28]), but it clearly does not indicate significant boron loss during prograde metamorphism. The retrograde metamorphism[6,29] common in parts of the Broken Hill block (Fig. 1) also has not substantially affected the boron isotopic composition of the tourmalines, as indicated by $\delta^{11}B$ values of −21.4‰ for metamorphically corroded tourmaline from a retrograde shear zone near the Nine Mile mine, and −20.1‰ for unaltered tourmaline from the same area but outside the shear zone. The −1.3‰ shift in $\delta^{11}B$ for this corroded tourmaline is in agreement with predicted isotope behaviour at high temperatures (M.R.P. and D. London, unpublished results), but is not significant relative to the range of $\delta^{11}B$ values measured for Broken Hill. We conclude that the mechanical and chemical stability of tourmaline, together with the lack of other boron-rich mineral hosts in the district, prevented major boron isotope fractionation during metamorphism in the Broken Hill block. This interpretation is consistent with tourmaline $\delta^{11}B$ data from greenschist-, amphibolite-, and granulite-facies terranes elsewhere[16].

Our model, based on boron isotope and other data, suggests that parts of the Thackaringa Group originally contained non-marine evaporites. Borates within such evaporites provide a source of abundant, readily extractable boron; concentrations of saline minerals and carbonates are also likely to have been present in such a setting. Deposition of the overlying Broken Hill Group in a shallow marine basin[3,7] was apparently associated with continental rifting and the development of a convecting hydrothermal system that extended into the underlying sediments of the Thackaringa Group, leaching them of boron, salts and, perhaps, sulphur and metals. The major stratabound lead-zinc-silver ores were then deposited by exhalative and/or diagenetic replacement processes together with siliceous lode rocks and manganese-iron sediments (such as garnet quartzites); coeval tourmalinites and stratabound tungsten mineralization formed generally in distal sedimentary environments. Widespread tourmalinites in the Broken Hill block, and the abundance of carbonate gangue in the main lead-zinc-silver lodes, may be explained by a source reservoir of non-marine evaporitic borates and carbonates in the Thackaringa Group. We suggest that the restriction of abundant carbonate to the lode horizons[9-11] in the upper Broken Hill Group reflects boiling of carbon-dioxide-rich hydrothermal fluids in a shallow marine basin, in which the carbon dioxide was derived by leaching of carbonate-bearing evaporites in the underlying Thackaringa Group. Evaporite salts in the Thackaringa Group leached by convecting hydrothermal fluids and resultant increased metal-chloride complexing may have been critically important for transporting the huge quantities of lead, zinc and silver deposited in the main lodes.

A possible modern analogue of the Broken Hill block is the Salton Sea geothermal field in southern California where deep geothermal fluids that interact with Pliocene lacustrine and non-marine evaporite sequences[30,31] have high concentrations of sodium, calcium, potassium, iron, manganese, zinc, lead, chlorine, lithium and boron[32]. This geothermal system is actively depositing iron-zinc-lead-copper sulphide minerals with $\delta^{34}S$ values from −9 to +9‰ (similar to sulphides of the Broken Hill lode[33,34]) which reflect sulphur derivation by the hydrothermal reduction of anhydrite-derived sulphate in evaporitic sediments at depth[35]. Continued rifting of the Salton Trough and transgression by sea water from the Gulf of California could produce a geological setting similar to that postulated for Broken Hill and an environment favourable for the formation of stratabound lead-zinc-silver mineralization and related tourmalinites. □

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1. Stevens, B. P. J. & Stroud, W. J. (eds) *Rec. Geol. Surv. New South Wales* **21** (1983).
2. Willis, I. L., Brown, R. E., Stroud, W. J. & Stevens, B. P. J. *Geol. Soc. Aust. J.* **30,** 195–224 (1983).
3. Stevens, B. P. J., Barnes, R. G., Brown, R. E., Stroud, W. J. & Willis, I. L. *Precambr. Res.* **40/41,** 297–327 (1988).
4. Marjoribanks, R. W., Rutland, R. W. R., Glen, R. A. & Laing, W. P. *Precambr. Res.* **13,** 209–240 (1980).
5. Phillips, G. N. *Contr. Miner. Petrol* **75,** 377–386 (1980).
6. Stevens, B. P. J. *Aust. J. Earth Sci.* **33,** 73–98 (1986).
7. Wright, J. V., Haydon, R. C. & McConachy, G. W. *Geology* **15,** 598–602 (1987).
8. Barnes, R. G. *Rec. Geol. Surv. New South Wales* **20,** 33–70 (1980).
9. Barnes, R. G. *Bull. Geol. Surv. New South Wales* **32,** 1–115 (1988).

10. Johnson, I. R. & Klingner, G. D. in *Aust. Inst. Min. Metall. Monogr.* Vol. 5 (ed. Knight, G. L.) 476–491 (1975).
11. Haydon, R. C. & McConachy, G. W. *Econ. Geol.* **82,** 826–856 (1987).
12. Slack, J. F. *Trans. Inst. Min. Metall.* **B91,** 81–89 (1982).
13. Taylor, B. E. & Slack, J. F. *Econ. Geol.* **79,** 1703–1726 (1984).
14. Slack, J. F., Herriman, N., Barnes, R. G. & Plimer, I. R. *Geology* **12,** 713–716 (1984).
15. Plimer, I. R. in *Base Metal Sulfide Deposits in Sedimentary and Volcanic Environments* (eds Friedrich, G. H. & Herzig, P. M.) 255–283 (Springer, Berlin, 1988).
16. Palmer, M. R. & Slack, J. F. *Contr. Miner. Petrol.* **103,** 431–451 (1989).
17. Palmer, M. R., Spivack, A. J. & Edmond, J. M. *Geochim. cosmochim. Acta* **51,** 2319–2323 (1987).
18. Spivack, A. J. & Edmond, J. M. *Geochim. cosmochim. Acta* **51,** 1033–1043 (1987).
19. Spivack, A. J., Palmer, M. R. & Edmond, J. M. *Geochim. cosmochim. Acta* **51,** 1939–1949 (1987).
20. Barnes, R. G. *et al. Rec. Geol. Surv. New South Wales* **21,** 289–323 (1983).
21. Plimer, I. R. in *Aust. Inst. Min. Metall.* **12,** 157–176 (1983).
22. Swihart, G. H. & Moore, P. B. *Geochim. cosmochim. Acta* **53,** 911–916 (1989).
23. Swihart, G. H., Moore, P. B. & Callis, E. L. *Geochim. cosmochim. Acta* **50,** 1297–1301 (1986).
24. Brown, R. E. *et al. Rec. Geol. Surv. New South Wales* **21,** 127–226 (1983).
25. Willis, I. L. thesis, Univ. New South Wales (1984).
26. Stroud, W. J. *et al.* Rec. Geol. Surv. New South Wales **21,** 227–287 (1983).
27. Barnes, R. G. *Geol. Surv. New South Wales Rep. GS 1980/116* (1980).
28. Ahmad, R. & Wilson, C. J. L. *Contr. Miner. Petrol.* **76,** 24–32 (1981).
29. Corbett, G. J. & Phillips, G. N. *Lithos* **14,** 59–73 (1981).
30. Herzig, C. T., Mehegan, J. M. & Stelting, C. E. *J. geophys. Res.* **93,** 12969–12980 (1988).
31. McKibben, M. A., Andes, J. P. Jr & Williams, A. E. *Econ. Geol.* **83,** 511–523 (1988).
32. Williams, A. E. & McKibben, M. A. *Geochim. cosmochim. Acta* **53,** 1905–1920 (1989).
33. Both, R. A. & Smith, J. W. *Econ. Geol.* **70,** 308–318 (1975).
34. Spry, P. G. *Min. Deposita* **22,** 109–115 (1987).
35. McKibben, M. A. & Eldridge, C. S. *Am. J. Sci.* **289,** 661–707 (1989).

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Wavelength sensitivity in blindsight

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BLINDSIGHT—the residual visual functions observed in visual-field defects resulting from destruction of part of the primary visual cortex (striate cortex) even though visual stimuli presented in the field defect are not consciously perceived—has generated new insights into the nature of consciousness and the role of the extrastriate pathways in visual processing. Some patients can detect and localize unseen stimuli when they are required to guess[1–4]. Discrimination of movement[5,6], flicker[5,7] and orientation[4,8] may also be present, but residual colour discrimination is controversial. Negative results (see ref. 9 for review) imply that only the pathways from eye to striate cortex can transmit information about colour in primates. By measuring sensitivity to light of different wavelengths in patients with blindsight we show that spectral sensitivity in the blind fields is surprisingly high, with a reduction of only 1 log unit or less across the visible spectrum. It is also essentially normal in form, whether the patients are adapted to light or dark. The shift in peak sensitivity from medium to shorter wavelengths in adaptation to the dark (the Purkinje shift) and the presence of discontinuities in the light-adapted curve together show that blindsight involves both rod and cone contributions, and that some colour opponency remains. As colour opponency requires input from primate beta retinal ganglion cells, two-thirds of which degenerate transneurally after a striate cortical lesion in juvenile monkeys[10], our results show that the surviving subpopulation of primate beta cells is functional.

The investigation of blindsight provides the only available behavioural method of isolating and studying the properties of the extra-geniculostriate cortical visual pathways in man, and can show which aspects of visual coding require the striate cortex and which do not. Damage to the striate cortex eventually leads to the death of the majority of ganglion cells in the corresponding part of the retina in man[11], and also in monkeys, where the degeneration is selective for, and involves up to 80% of, the colour opponent primate beta ($P\beta$) cells[10]. To see whether the pathways from the degenerated hemiretina can process wavelength information we measured spectral sensitivity in the normal and blind fields of three patients in whom blindsight had been previously demonstrated[9], and in two normal observers. We used both adaptation to the light and dark because the contradictory reports on colour discrimination in blindsight[4,5,7,9,12–16] include a demonstration that spectral sensitivity of totally destriated monkeys is characteristic of rod receptors regardless of adaptation level[15], indicating that only the colour-blind rod channel is effective.

The subjects were tested in a Tübinger perimeter, using monocular viewing such that their field defect was on the temporal side, corresponding to the normally more sensitive nasal retina. The field defects of the three patients are shown in Fig. 1. Stimuli were presented at positions 10° eccentric from the fixation axis, permitting a comparison of sensitivity at mirror-symmetric positions in the hemifields. While held by a head and chin rest, the subject fixated a central dim red point. The viewing eye was monitored through an infrared video system. Nine coloured targets (116′, 200 ms) from 450 to 660 nm were used. Each was produced by a narrowband, 9–13 nm, interference filter. In the normal visual hemifield detection thresholds were repeatedly measured by progressively increasing stimulus intensity by 0.05 log units until the subject reported seeing the target on three consecutive presentations. For the blind field a guessing paradigm was used. Blank trials (on which no target

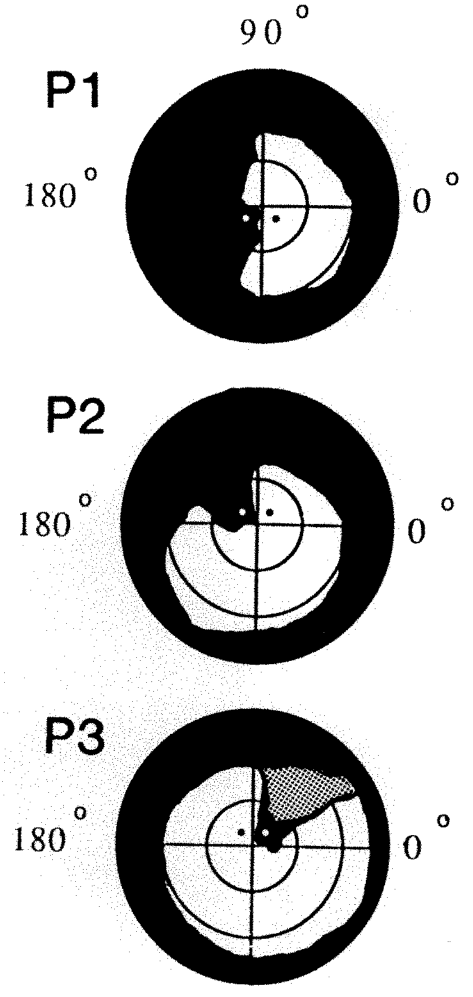

FIG. 1 Visual fields of patients plotted by moving a 320 cdm^{-2} 44′ white target on a 10 cdm^{-2} background from the periphery towards the fixation point. The stippled area in P3 was amblyopic, as revealed by stationary flashed targets. Black or white spots at 10° eccentricity indicate the position where coloured targets were presented in intact or blind fields respectively.

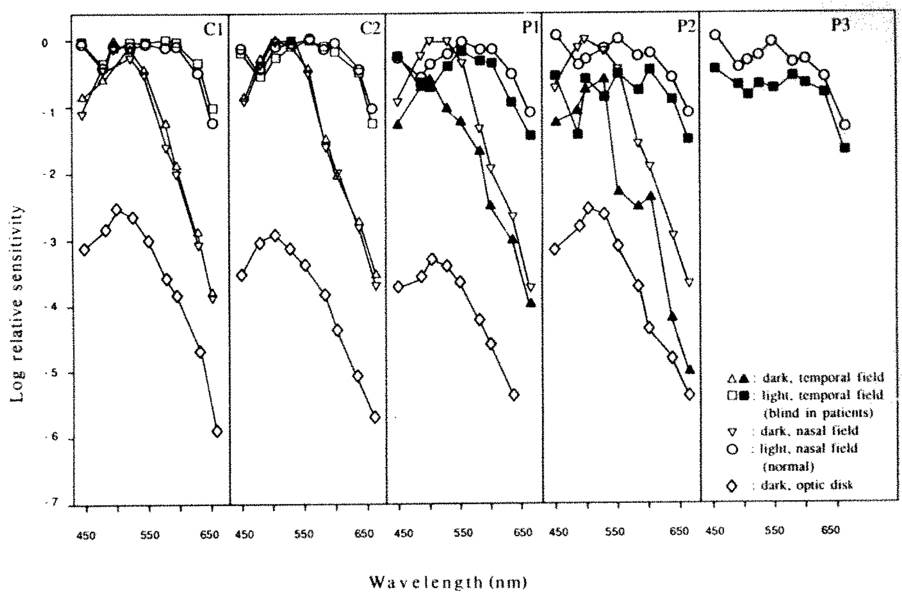

FIG. 2 Relative spectral sensitivity at an eccentricity of 10° in two normal subjects and in the patients whose field defects are shown in Fig. 1. In each graph the most sensitive dark-adapted value is scaled to zero. The light-adapted values have been raised by 4.3(C1), 3.9(C2) and 3.7(P1,P2) log units so that the envelopes of the light- and dark-adapted curves can be readily compared. The key to the symbols is below the curves for P3, who was not tested when dark adapted. Black symbols refer to blind fields.

was presented) and stimulus trials, with an overall ratio of 4:1, were presented randomly, and the patient was asked to guess whether a light had been presented. After each batch of up to 250 trials, target luminance was increased by 0.1 long units, until detection was statistically significant at the 5% level. If a different value was obtained when the measurement was repeated, the mean was used. Stimuli were presented on a white 3,500 ± 1,000 K background of low photopic (32 $\mathrm{cd\,m^{-2}}$) or scotopic luminance (0.0001 $\mathrm{cd\,m^{-2}}$) after 20 min of adaptation to the dark. Under scotopic conditions, straylight detection thresholds were determined by presenting the targets in the natural blind spots; under photopic conditions the targets could not be made intense enough to be detectable in the blind spot.

Figure 2 shows the spectral sensitivity curves. Note that sensitivity in the normal and blind fields has a single peak at 500 nm under adaptation to the dark, the smooth curve and steep decline above 550 nm being characteristic of rod vision. Sensitivity in the blind fields is normal, apart from being reduced by up to a long unit, and the curves are less smooth. Comparison with the straylight detection thresholds measured under adaptation to the dark for targets on the reflective natural blind spot, which are 2-3 log units higher than for nearby targets in normal retina, shows that as sensitivity in the blind field is reduced by not more than 1 log unit it is not based on the detection of light scattered onto the functionally normal retina. Under adaptation to the light there is no steep decline in sensitivity above 550 nm such as occurs under scotopic conditions; instead, the curve shifts towards the longer wavelengths, showing that cones are involved.

Whereas the sensitivity of the colour-opponent system, especially when tested with targets on a high photopic white surround, has peaks at about 450, 525–550, and 600 nm, and notches at 480 and 580 nm (refs 17–19) (their precise positions depending on intensity and colour temperature of the background) the luminance system has a single broad peak around 555 nm (ref. 19). Given that the more sensitive system will determine threshold, we assume some contribution of the luminance system in governing sensitivity around 550 nm that is in accordance with the lack of a clear notch at 580 nm in several photopic curves. The sensitivity of the luminance system, however, decreases smoothly towards the short wavelengths[19], probably because the broadband channels receive little if any input from short wavelength ('blue') cones[20,21], and can therefore not be responsible for the prominent secondary increase in sensitivity from 480/500 nm to 450 nm. The presence of this increase in all photopic curves indicates that colour opponent processes contribute to or even determine thresholds for the 450 nm blue stimulus. This is supported by the results of colour naming at threshold in the good field, which was faultless for the blue and red, but uncertain and occasionally wrong for green and yellow stimuli.

Together, our results show that spectral sensitivity as measured under the present conditions differs between normal and cortically blind hemifields only in terms of absolute sensitivity. This difference is fairly small and is not an artefact of comparing different detection methods because it was not altered when the same guessing paradigm was applied in the normal field. There is no evidence that sensitivity is mediated solely by rod receptors, as this would abolish the marked difference between curves measured under adaptation to the light and dark. All measurements made under photopic conditions show contributions from cone receptors. The notch or shoulder at 580 nm and the rise in sensitivity from 480 to 450 nm provide evidence for colour opponent processes. If chromatic opponency does remain in blindsight, genuine wavelength discrimination may also be possible. This can now be tested by presenting coloured stimuli that are matched for luminous efficiency on the basis of the patient's own spectral sensitivity. Thus far, evidence indicates that the subpopulation of surviving $P\beta$ ganglion cells, which are the only known ganglion cells to provide colour-opponent signals, is still functional. As chromatic opponency has never been detected in the midbrain visual pathway (a favourite candidate for the mediation of blindsight) remaining retinal projections to the thalamus and from there to extrastriate cortex may be involved[22,23]. □

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1. Perenin, M. T. & Jeannerod, M. *Neuropsychologia* **16,** 1–13 (1978).
2. Pöppel, E., Held, R. & Frost, D. *Nature* **243,** 295–296 (1973).
3. Stoerig, P. & Pöppel, E. *Expl Brain Res.* **64,** 469–475 (1986).
4. Weiskrantz, L., Warrington, E. K., Sanders, M. D. & Marshall, J. *Brain* **97,** 709–728 (1973).
5. Barbur J. L., Ruddock, K. H. & Waterfield, V. A. *Brain* **103,** 905–928 (1980).
6. Weiskrantz, L. *Blindsight* (Clarendon, Oxford, 1986).
7. Blythe, I. M., Kennard, C. & Ruddock, K. H. *Brain* **110,** 887–905 (1987).
8. Perenin, M. T. *Neuropsychologia* **16,** 697–708 (1978).
9. Stoerig, P. *Brain* **110,** 869–886 (1987).
10. Cowey, A., Stoerig, P. & Perry, V. H. *Neuroscience* **29,** 65–80 (1989).
11. Van Buren, J. M. *The Retinal Ganglion Cell Layer* (Charles Thomas, Springfield, 1963).
12. Bender, M. B. & Krieger, H. P. *Archs. Neurol. Psychiat., Chicago* **65,** 72–79 (1951).
13. Keating, E. G. *Brain Res.* **179,** 379–384 (1979).
14. Schilder, P., Pasik, P. & Pasik, T. *Expl Brain Res.* **14,** 436–448 (1972).
15. Leporé, F., Cardu, B., Rasmussen, T. & Malmo, R. B. *Brain Res.* **93,** 203–221 (1975).
16. Klüver, H. *J. Psychol.* **11,** 23–45 (1941).
17. Snelgar, R. S., Foster, D. H. & Scase, M. O. *Vision Res.* **27,** 1017–1027 (1987).
18. Sperling, H. G. & Harwerth, R. S. *Science* **172,** 180–184 (1971).
19. King-Smith, P. E. & Carden, D. *J. opt. Soc. Am.* **66,** 709–717 (1976).

20. Gouras, P. *J. Physiol., Lond.* **199**, 533–547 (1968).
21. Lee, J. & Strohmeyer, C. F. *J. Physiol., Lond.* **413**, 563–593 (1989).
22. Cowey, A. & Stoerig, P. *Expl Brain Res.* **75**, 631–638 (1989).
23. Felsten, G., Benevento, L. A. & Burman, D. *Brain Res.* **288**, 363–367 (1983).

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Arachidonic acid induces a prolonged inhibition of glutamate uptake into glial cells

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ACTIVATION of NMDA (*N*-methyl-D-aspartate) receptors by neurotransmitter glutamate stimulates phospholipase A_2 to release arachidonic acid[1,2]. This second messenger facilitates long-term potentiation of glutamatergic synapses in the hippocampus[3], possibly by blocking glutamate uptake[4,5]. We have studied the effect of arachidonic acid on glutamate uptake into glial cells using the whole-cell patch-clamp technique to monitor the uptake electrically[6,7]. Micromolar levels of arachidonic acid inhibit glutamate uptake, mainly by reducing the maximum uptake rate with only small effects on the affinity for external glutamate and sodium. On removal of arachidonic acid a rapid (5 minutes) phase of partial recovery is followed by a maintained suppression of uptake lasting at least 20 minutes. Surprisingly, the action of arachidonic acid is unaffected by cyclo-oxygenase or lipoxygenase inhibitors suggesting that it inhibits uptake directly, possibly by increasing membrane fluidity. As blockade of phospholipase A_2 prevents the induction of long-term potentiation (LTP)[8], inhibition of glutamate uptake by arachidonic acid may contribute to the increase of synaptic gain that occurs in LTP. During anoxia, release of arachidonic acid[9] could severely compromise glutamate uptake and thus contribute to neuronal death.

Application of L-glutamate to glial (Müller) cells from the salamander retina evokes an inward current reflecting glutamate uptake by an electrogenic carrier that transports Na^+ into the cell[6,7]. A response with similar characteristics has recently been demonstrated in mammalian glial cells[10,11]; we used salamander glia for this study because of the large uptake current they exhibit. Glutamate uptake was monitored as the inward current evoked by 30 μM glutamate in isolated glial cells voltage-clamped to −40 mV. Arachidonic acid produced a slowly developing inhibition of glutamate uptake (Fig. 1*a*–*c*). The uptake current was halved by 2 minutes' exposure to 10 μM arachidonic acid, and significant inhibition was seen with 1 μM arachidonic acid (Fig. 1*d*).

The dependence of the uptake current on external glutamate and external sodium concentrations, measured before and after applying arachidonic acid, showed that arachidonic acid suppresses uptake largely by reducing the maximum uptake rate, with a small increase in the apparent affinity for glutamate and a small decrease in the apparent affinity for external sodium (Fig. 2*a*, *b*).

The mechanism of action of arachidonic acid was investigated pharmacologically. Ouabain (10^{-4} M) did not prevent the inhibition produced by arachidonic acid (Fig. 3*a*), which was therefore not an indirect result of intracellular Na^+ accumulation following inhibition of the sodium pump by arachidonic acid[4]. The cyclo-oxygenase inhibitor indomethacin (5 μM) had no effect on the uptake inhibition (Fig. 3*b*), ruling out the possibility that it depends on the conversion of arachidonic acid to prostaglandin derivatives. Nordihydroguaiaretic acid (NDGA, 10 μM), a lipoxygenase inhibitor, produced a slowly developing inhibition of uptake similar to that seen with arachidonic acid (possibly by inhibiting breakdown of arachidonic acid produced by the glial cell itself), but did not prevent inhibition of uptake by arachidonic acid (Fig. 3*c*). Metabolites of arachidonic acid like HPETE[12] are therefore not responsible for the inhibition of

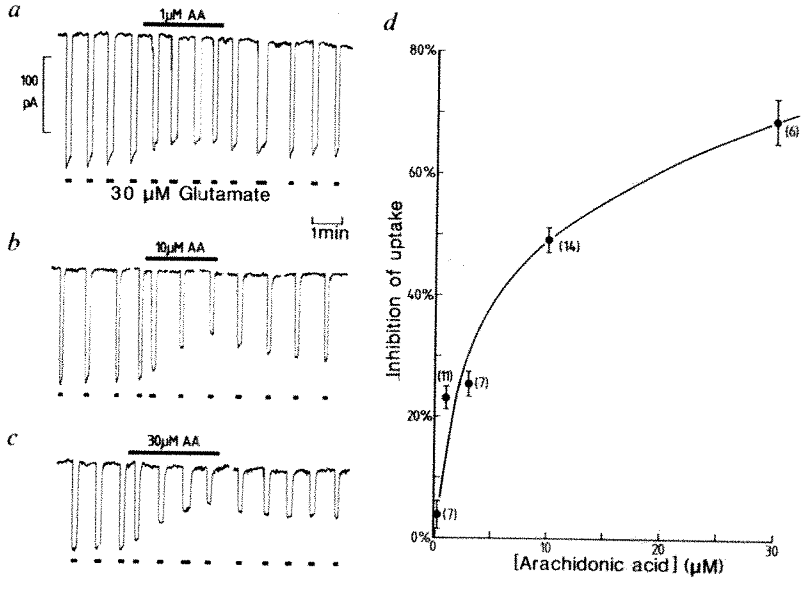


FIG. 1 Arachidonic acid inhibits glial cell glutamate uptake. *a*, *b* and *c*, current changes in three different Müller cells voltage-clamped to −40 mV during repeated application of glutamate (30 μM) (timing shown by black bars below each record). Glutamate evokes an inward current (downward deflection) reflecting electrogenic glutamate uptake[6]. Application of 1 μM (*a*), 10 μM (*b*) or 30 μM (*c*) arachidonic acid produces a slowly developing inhibition of the glutamate-evoked current. *d*, Percentage suppression of glutamate uptake (mean ± s.e.) as a function of arachidonic acid concentration measured after 2 min in the presence of arachidonic acid (number of cells shown by each point; smooth curve drawn by eye). During prolonged recordings even in the absence of arachidonic acid there is a slow decay of glutamate-evoked current, typically 2% per min, which correlates with a decrease in cell capacitance (measured from the current response to voltage jumps). To compensate for this loss of cell area, which would otherwise lead to an overestimate of the suppressive effect of arachidonic acid, glutamate-evoked currents were normalized by the cell capacitance measured before and in the presence of arachidonic acid. Normalized uptake current declined much more slowly over a prolonged recording period than did the un-normalized current (see Fig. 4).

METHODS. Glial (Müller) cells isolated from the tiger salamander retina[6] were whole-cell clamped using pipettes containing (mM): KCl, 95; HEPES, 5; NaCl, 5; Na_2ATP, 5; $MgCl_2$, 7; $CaCl_2$, 1; K_2EGTA, 5; the pH was adjusted to 7.0 with 14 mM KOH. External solution contained (mM): NaCl, 105; KCl, 2.5; $CaCl_2$, 3; $MgCl_2$, 0.5; glucose, 15; HEPES, 5; $BaCl_2$ (to block the cells' potassium conductance), 6. The pH was adjusted to 7.3 with 2 mM NaOH. Temperature, 20–23 °C. Arachidonic acid (Sigma) was stored under nitrogen at −20 °C as a 10 mM stock suspension in water or occasionally in DMSO, which gave similar results. When required this stock was thawed, sonicated for 5 min, and added to the superfusion solution, which was then sonicated for 5 min.

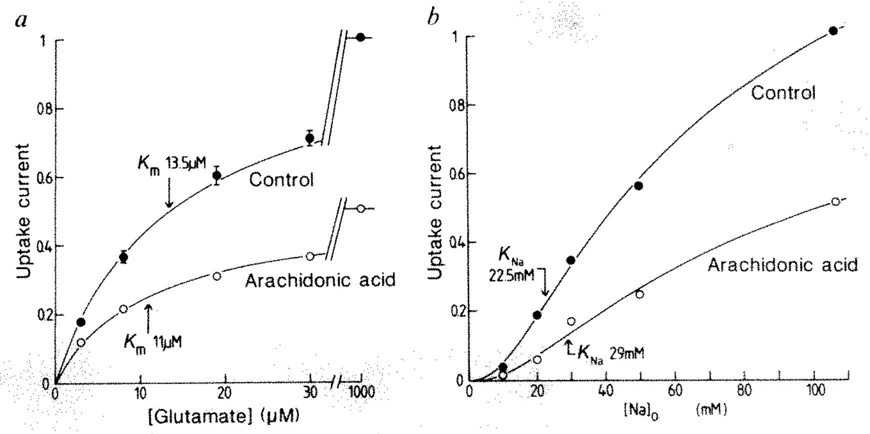


FIG. 2 Effect of arachidonic acid on the glutamate- and sodium-dependence of glutamate uptake. *a*, Dependence of uptake current (at −40 mV) on glutamate concentration before (●) and after (○) 3 min in the presence of 10 μM arachidonic acid. All data normalized to the saturating response before arachidonic acid. Mean (±s.e.) data from 10, 11, 5 and 6 cells for 3, 8, 19 and 30 μM glutamate. Curves through the points are Michaelis–Menten relations with $K_m = 13.5$ μM, $V_{max} = 1$ before, and $K_m = 11$ μM, $V_{max} = 0.5$ after arachidonic acid. Arachidonic acid halves the V_{max} of uptake with a small increase in apparent glutamate affinity. *b*, External sodium-dependence of the uptake current (at −40 mV) evoked by 30 μM glutamate before (●) and after (○) 3 min in 10 μM arachidonic acid. Data normalized to control response in 107 mM $[Na]_o$. Mean data (s.e.< symbol size) from 3, 5, 11 and 5 cells in 10, 20, 30 and 50 mM $[Na]_o$. Smooth curves are empirical fits with the form: $V_{high\,Na}\{[Na]_o/([Na]_o + K_{Na})\}^3$, with $K_{Na} = 22.5$ mM, $V_{high\,Na} = 1.77$ before, and $K_{Na} = 29$ mM, $V_{high\,Na} = 1.05$ after arachidonic acid. On this basis, most of the uptake inhibition produced by arachidonic acid in 107 mM $[Na]_o$ in Fig. 1 results from a decrease in the rate of uptake that would be observed in saturating $[Na]_o$, although there is some contribution from the decrease in apparent affinity for Na_o. Fitting the data with the Hill equation instead of a power of the Michaelis–Menten relation led to the same conclusion.

glutamate uptake. Oleic acid inhibited uptake approximately half as well as arachidonic acid (Fig. 3*d*). Phorbol ester, which stimulates protein kinase C by 20–60%[13], had little effect on uptake (Fig. 3*e*), ruling out the possibility that arachidonic and oleic acids reduce uptake by stimulating this kinase[13]. The longer *cis*-unsaturated fatty acid docosahexaenoic acid, which has no effect on protein kinase C[13], produced an inhibition of uptake similar to that produced by arachidonic acid, and the protein kinase inhibitor H-7 had no effect on the action of arachidonic acid (see Fig. 3*e* legend).

To test whether the effects of arachidonic and oleic acids are a consequence of the lipid disruption produced by the kinked *cis* double bonds in their structures (20:4 and 18:1 respectively), we applied two linear analogues of these molecules. Elaidic acid (18:1), a homologue of oleic acid with a *trans* instead of a *cis* double bond, produced only one quarter of the uptake inhibition produced by oleic acid (Fig. 3*f*). Similarly arachidic acid (20:0), the saturated analogue of arachidonic acid, produced only about one fifth of the inhibition that arachidonic acid did (Fig. 3*g*).

These results are consistent with the inhibition of uptake produced by arachidonic acid being due to the disruption of membrane lipid organization (increase in membrane fluidity) caused by the introduction of unsaturated fatty acid into the membrane. The messenger actions of arachidonic acid are usually attributed to its lipoxygenase and cyclo-oxygenase derivatives. The direct action of arachidonic acid we propose here is similar to that suggested for the effect of arachidonic acid on the sodium pump[14]. Changes of membrane fluidity have also been proposed to regulate phosphate and glucose uptake in the kidney[15].

The kinetics of action of arachidonic acid are of interest with respect to the possible role of arachidonic acid in long-term

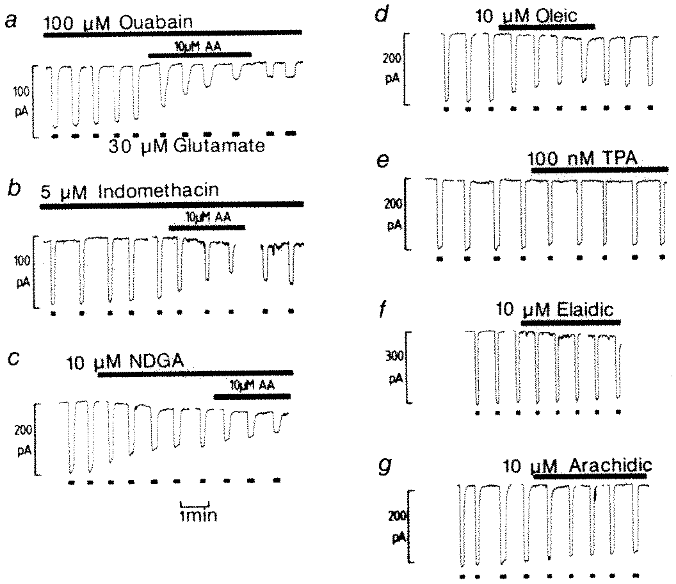


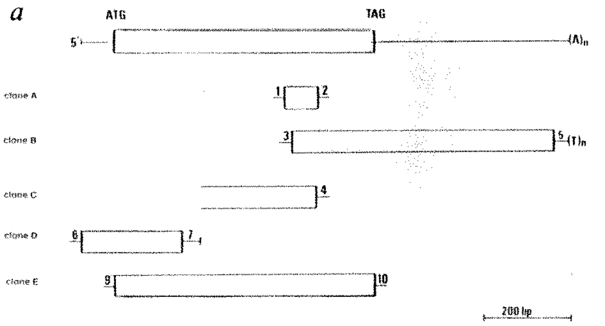
FIG. 3 Pharmacology of the uptake inhibition. Uptake measured as the current (normalized by capacitance for quantitative analysis, see Fig. 1) evoked at −40 mV by 30 μM glutamate. *a*, Ouabain does not prevent the uptake inhibition produced by 10 μM arachidonic acid. Mean (±s.e.) inhibition produced in three cells by 2 minutes' arachidonic acid in the presence of 10^{-4} M ouabain was 55.6 ± 2.7%, similar to that seen without ouabain (Fig. 1). *In vivo*, where Na_i is not controlled by the patch pipette, accumulation of Na^+ inside the cell, following inhibition of the sodium pump[4] by arachidonic acid, may contribute to inhibition of glutamate uptake. *b*, Indomethacin (5 μM, for 25 min) does not prevent the uptake inhibition produced by 10 μM arachidonic acid. Mean inhibition produced by 2 minutes' arachidonic acid after 20–30 min in indomethacin was 47.9 ± 5.3% ($n = 3$), similar to that without indomethacin. In toad bladder, 6 μM indomethacin inhibits cyclo-oxygenase by >90%[21]. *c*, Nordihydroguaiaretic acid (NDGA, 10 μM) does not prevent the uptake inhibition produced by 10 μM arachidonic acid. NDGA itself reduced the uptake current (by 52.8 ± 2.1% after 8 min in NDGA, $n = 9$), and subsequent application of arachidonic acid further reduced uptake by 45.1 ± 4.7% ($n = 10$, after 2 min), similar to the inhibition seen without NDGA. Estimates of the 50% inhibitory concentration for NDGA block of lipoxygenase in rat and human are ~0.9 μM and ~3 μM, respectively[22,23]. *d*, Oleic acid, with one *cis* double bond in its structure, unlike the four in arachidonic acid, reduces uptake, but to a lesser degree than arachidonic acid. Mean reduction produced by 2 min of 10 μM oleic acid was 28.9 ± 1.0% (seven cells). *e*, Arachidonic and oleic acids do not act by stimulating protein kinase C. Phorbol 12-myristate 13-acetate (TPA, 100 nM), which activates protein kinase C by 20–60%[13], has little effect on uptake (mean reduction in five cells after 4.5 min in TPA was 5.5 ± 0.9%). Similar results were obtained with 10^{-6} M pipette $[Ca^{2+}]$, rather than 10^{-7} M $[Ca^{2+}]_{pipette}$ as here. Furthermore, docosahexaenoic acid (22:6, 10 μM for 2 min), which has no effect on protein kinase C[13], reduced the uptake current by 58.9 ± 2.6% ($n = 5$, data not shown). Including the protein kinase inhibitor H7 (340 μM) in the patch pipette had no effect on the uptake inhibition produced by arachidonic acid in five cells. *f*, Elaidic acid has much less effect on uptake than does its *cis* homologue oleic acid. Mean reduction of uptake after 2 min in 10 μM elaidic acid was 6.7 ± 1.8% (nine cells). *g*, Arachidic acid has much less effect on uptake than does its polyunsaturated analogue arachidonic acid. Mean reduction of uptake after 2 min in 10 μM arachidic acid was 10.4 ± 1.3% (eight cells). Drugs were made up as stock solutions in dimethylsulphoxide (DMSO) (0.1% final concentration; DMSO was also added to control solutions). Gaps in the records are where voltage pulses were applied to measure cell capacitance.

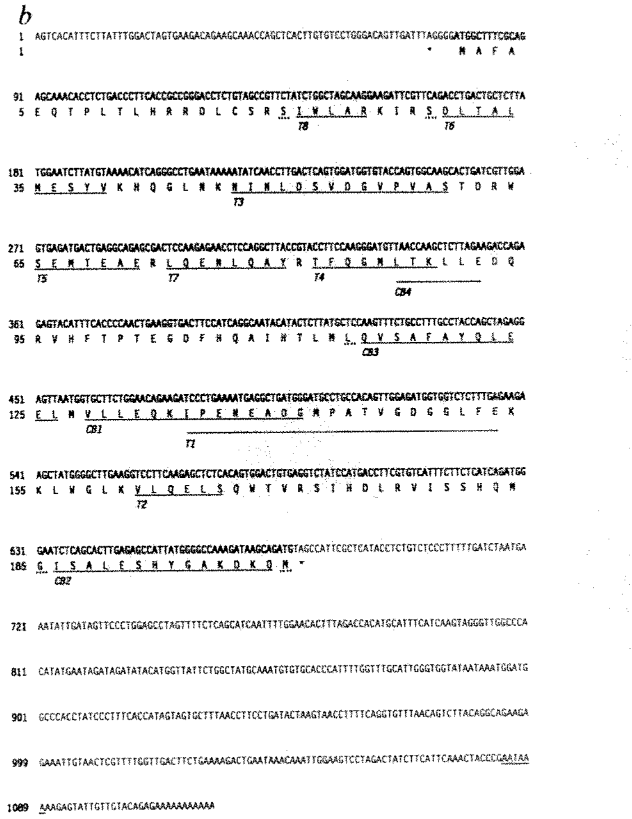
expression, developmental regulation and localization. The complementary DNA-deduced amino-acid sequence and subsequent expression of cDNA clones covering the entire coding region in HeLa-cells indicate that CNTF is a cytosolic protein. This, together with its regional distribution and its developmental expression, show that CNTF is not a target-derived neurotrophic factor. CNTF thus seems to exhibit neurotrophic and differentiation properties only after becoming available either by cellular lesion or by an unknown release mechanism.

CNTF was purified from adult rat sciatic nerve as described previously[5], using an additional HPLC purification step (for details see legend to Table 1). The amino-acid sequences of the various fragments determined by gas-phase microsequencing represented more than 50% of the protein. The peptide sequences thus obtained matched perfectly with those deduced from cDNA cloning as shown in Fig. 1.

To obtain RNA for molecular cloning, we used cultures of rat brain cells that previously had been shown to produce substantial quantities of CNTF (ref. 7). After various polymerase chain reaction steps, the nucleotide sequences of clones A, B, C revealed a short 5′ untranslated region of 77 base pairs (bp) and an open reading frame of 600 bp, predicting a protein 200 amino acids long with a 3′ untranslated region of 436 bp, which ends in a poly(A) tail (Fig. 1*b*). The initiation site for translation was localized at position 78–80 of the nucleotide sequence. A stop codon located 5′ to this initiation site at position 72–74 and G residues in positions 75 and 81 fulfil the requirements for an adequate translation initiation site, according to Kozak[8].

Although the dibasic (Arg–Arg) sequence in positions 13 and 14 of the predicted sequence represents a potential post-translational cleavage site, the amino-acid composition of purified CNTF (Table 1) does not favour such a cleavage: the amino acids phenylalanine, arginine and alanine present in the N-terminal region are not reduced relative to other amino acids that are absent from this region (for example, isoleucine). Moreover, the predicted relative molecular mass (M_r) of 22,800 (22.8K), corresponding to the 200-amino-acid sequence is in complete agreement with that estimated from PAGE analysis (22.5K) (ref. 6). Thus, the amino-acid sequence of CNTF shows the features of a cytosolic protein; that is, no signal peptide, no consensus sequences for glycosylation and only one cysteine residue at position 17. Comparison of the determined CNTF sequence with those of the PIR and EMBL databases did not reveal significant similarities with any other known protein. In




b
1 AGTCACATTTCTTATTTGGACTAGTGAAGACAGAAGCAAACCAGCTCACTTGTGTCCTGGGACAGTTGATTTAGGGGATGGCTTTCGCAG
1 * M A F A
91 AGCAAACACCTCTGACCCTTCACCGCCGGGACCTCTGTAGCCGTTCTATCTGGCTAGCAAGGAAGATTCGTTCAGACCTGACTGCTCTTA
5 E Q T P L T L H R R D L C S R S I W L A R K I R S D L T A L
T8
T6
181 TGGAATCTTATGTAAAACATCAGGGCCTGAATAAAAATATCAACCTTGACTCAGTGGATGGTGTACCAGTGGCAAGCACTGATCGTTGGA
35 M E S Y V K H Q G L N K N I N L D S V D G V P V A S T D R W
T3
271 GTGAGATGACTGAGGCAGAGCGACTCCAAGAGAACCTCCAGGCTTACCGTACCTTCCAAGGGATGTTAACCAAGCTCTTAGAAGACCAGA
65 S E M T E A E R L Q E N L Q A Y R T F Q G M L T K L L E D Q
T5
T7
T4
CB4
361 GAGTACATTTCACCCCAACTGAAGGTGACTTCCATCAGGCAATACATACTCTTATGCTCCAAGTTTCTGCCTTTGCCTACCAGCTAGAGG
95 R V H F T P T E G D F H Q A I H T L M L Q V S A F A Y Q L E
CB3
451 AGTTAATGGTGCTTCTGGAACAGAAGATCCCTGAAAATGAGGCTGATGGGATGCCTGCCACAGTTGGAGATGGTGGTCTCTTTGAGAAGA
125 E L M V L L E Q K I P E N E A D G M P A T V G D G G L F E K
CB1
T1
541 AGCTATGGGGCTTGAAGGTCCTTCAAGAGCTCTCACAGTGGACTGTGAGGTCTATCCATGACCTTCGTGTCATTTCTTCTCATCAGATGG
155 K L W G L K V L Q E L S Q W T V R S I H D L R V I S S H Q M
T2
631 GAATCTCAGCACTTGAGAGCCATTATGGGGCCAAAGATAAGCAGATGTAGCCATTCGCTCATACCTCTGTCTCCCTTTTTGATCTAATGA
185 G I S A L E S H Y G A K D K Q M *
CB2
721 AATATTGATAGTTCCCTGGAGCCTAGTTTTCTCAGCATCAATTTTGGAACACTTTAGACCACATGCATTTCATCAAGTAGGGTTGGCCCA
811 CATATGAATAGATAGATATACATGGTTATTCTGGCTATGCAAATGTGTGCACCCATTTTGGTTTGCATTGGGTGGTATAATAAATGGATG
901 GCCCACCTATCCCTTTCACCATAGTAGTGCTTTAACCTTCCTGATACTAAGTAACCTTTTCAGGTGTTTAACAGTCTTACAGGCAGAAGA
999 GAAATTGTAACTCGTTTTGGTTGACTTCTGAAAAGACTGAATAAACAAATTGGAAGTCCTAGACTATCTTCATTCAAACTACCCGAATAA
1089 AAAGAGTATTGTTGTACAGAGAAAAAAAAAAA

c

Oligo number	Sequence (5′-3′)	orientation	position
1	cccgggaattcGARCARAARATHCC	sense	468-481
2	ctgcagatatcTTYTCRAANARNCC	antisense	525-538
3	ctgctagagtcgacaagcttTGAAAATGAGGCTGATGGGATG	sense	482-503
4	cgtctagagtcgacaagcttACCATCTCCAACTGTGGCAGG	antisense	504-524
5	cggatccgaattctgcagTTTTTTTTTTTTTV	antisense	A TAIL
6	ccggatccgcggccgCCCCCCCCCCCCCC	sense	G TAIL
7	cctctagactcgaGTGCTTGCCACTGGTAC	antisense	243-263
8	AATATCAACCTTGACTCAGTGGAT	sense	215-238
9	cctctagaATGGCTTTCGCAGAGCAAACA	sense	78- 98
10	cctctagaCATCTGCTTATCTTTGGCCCC	antisense	657-677
11	GARAAYGARGCIGAYGGIATGCC	sense	483-505

FIG. 1 *a*, Complementary DNA cloning, and *b*, nucleotide and deduced amino-acid sequence of CNTF. The oligonucleotides used as primers are shown in *c* and their corresponding positions in the nucleotide sequence in *b*. Multiple cloning sites are indicated as lower-case letters. In the case of degenerated codons we used IUPAC symbols. Underlined amino acids in *b* correspond to the peptide sequences obtained from tryptic (T1-T8) and cyanogen bromide (CBI-4) fragments of CNTF. At the nucleotide level, sequences corresponding to the coding region are bold-faced and the polyadenylation signal sequence is underlined.

METHODS. Cultured rat astrocytes were grown for 3 weeks as described[20]. One day after final subculturing, total RNA was extracted from the cells[21]. Complementary DNA was synthesized using oligonucleotide 5 and reverse transcriptase of the Moloney murine leukaemia virus (Bethesda Research Laboratories). The first strand of cDNA served as a template for amplification of specific segments of CNTF using PCR[22]. Clone A was generated using the degenerated primer-oligos 1 and 2; the PCR product was identified with oligonucleotide 11, subcloned and sequenced by the dideoxynucleotide chain-termination method[23]. The nucleotide sequence thus obtained was used to synthesize primers 3 and 4 for amplification of cDNA ends according to Frohmann *et al.*[24] The cDNAs obtained were subcloned into the Bluescript SK+ vector (Stratagene) and sequenced (clones B and C). Oligonucleotide 7 was derived from the sequence of a genomic clone (Carroll, unpublished data). This primer, together with oligonucleotide 6 was used to create clone D using essentially the same protocol as for clone C, except that we performed a G-tailing procedure instead of T-tailing to generate 5′clones[25]. The inserts of several clones were sequenced and shown to be identical. PCR with newly synthesized cDNA from astrocytes and oligonucleotides 9 and 10 revealed a cDNA clone covering the entire coding region. The cDNA thus obtained (Fig. 1*a*, clone E) was used for expression in eukaryotic cells.

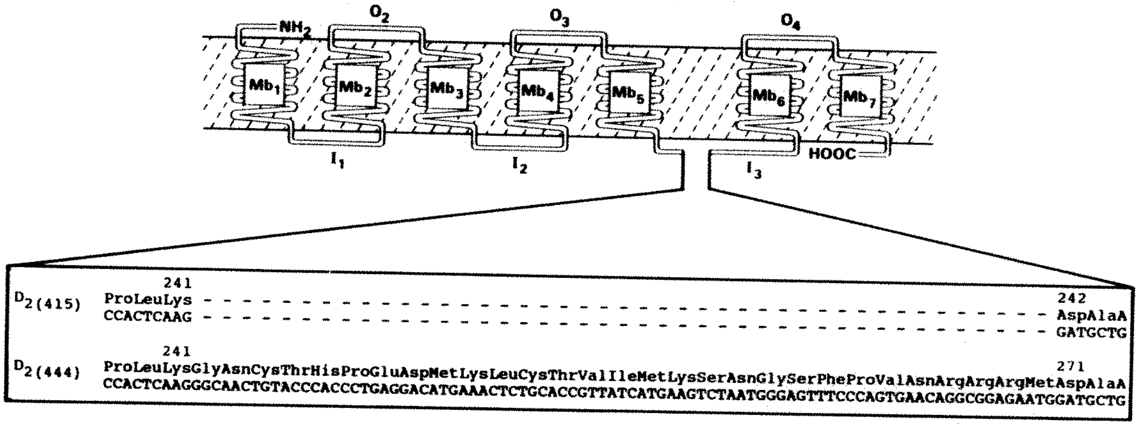

NH2
O2
O3
O4
Mb1
Mb2
Mb3
Mb4
Mb5
Mb6
Mb7
I1
I2
I3
HOOC
D2(415)
241
ProLeuLys
CCACTCAAG
242
AspAlaA
GATGCTG
D2(444)
241
ProLeuLysGlyAsnCysThrHisProGluAspMetLysLeuCysThrValIleMetLysSerAsnGlySerPheProValAsnArgArgArgMetAspAlaA
271
CCACTCAAGGGCAACTGTACCCACCCTGAGGACATGAAACTCTGCACCGTTATCATGAAGTCTAATGGGAGTTTCCCAGTGAACAGGCGGAGAATGGATGCTG

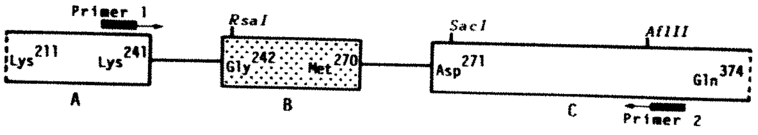
FIG. 1 Schematic representation of the structure of two isoforms of the D_2 dopamine receptor. These two isoforms, termed $D_{2(415)}$ and $D_{2(444)}$, differ in that the latter has a predicted 29-amino-acid sequence located within the putative third cytoplasmic loop between Lys 241 and Asp 242 (nucleotides 723 and 724). Note the presence of two potential glycosylation sites at Asn 243 and Asn 260. Otherwise the nucleotide sequence of the two clones is identical to that established by Bunzow *et al.*[7].

METHODS. Overlapping oligonucleotides were synthesized (Clontech) from the sequence of D_2 dopamine receptor cDNA (nucleotides 146–233 in ref. 7), radiolabelled by 'filling reaction' using *Escherichia coli* DNA polymerase I (Klenow fragment; Boehringer) and used to screen an oligo(dT)-primed cDNA library constructed in λgt10 (refs 20 and 21) from total rat brain poly(A)$^+$ RNA[22,23]. After transfer of the phage onto nitrocellulose filters (BA 85; Schleicher & Schuell), they were hybridized at 42 °C for 16 h in a buffer (6 × SSC, 0.05% sodium pyrophosphate, 8 mM Tris-HCl buffer, pH 7.4, 20% formamide, 1 × Denhardt's solution, 100 μg ml^{-1} yeast transfer RNA and 20 μg ml^{-1} salmon sperm DNA) containing 5×10^5 d.p.m. of the synthetic probe per ml. Filters were washed twice for 20 min at 25 °C in 2 × SSC, 0.05% sodium pyrophosphate, 0.1% SDS and, twice for 30 min at 37 °C in 0.2 × SSC, 0.1% SDS, 0.05% sodium pyrophosphate. *Eco*RI inserts of positive clones were subcloned in M13 mp18 or mp19 vectors (Boehringer) for sequencing[24] and in pGEM-4Z (Promega Biotec) for restriction analysis and preparation of fragments for use as probes.

protein with a total of 444 amino acids: the $D_{2(444)}$ receptor (Fig. 1). The sequences otherwise are 100% homologous in their coding regions and with the cDNA previously described[7].

We analysed rat genomic DNA to confirm that the $D_{2(415)}$ and $D_{2(444)}$ receptors are products of the same gene. First, Southern blot analysis indicated the presence of unique *Bam*HI and *Eco*RI restriction fragments, of 7.5 and 4.5 kilobases (kb) respectively, when probed with the *Sac*I–*Afl*II fragment (Fig. 2), which corresponds to a part of the third cytoplasmic loop common to both $D_{2(415)}$ and $D_{2(444)}$ receptors (data not shown). We also screened a genomic library with a *Bam*HI–*Bgl*II probe containing the full coding sequence of the cDNA for the $D_{2(444)}$ receptor (nucleotides 4 to 1,319), and then selected 21 clones out of 10^6 that were either identical or overlapping, using restriction enzyme digestion and Southern blot analysis. All of these clones hybridized strongly to an *Rsa*I–*Sac*I probe, corresponding mainly to the 29-amino-acid fragment characteristic of the $D_{2(444)}$ receptor (Fig. 2). Partial sequencing of these clones revealed the complex organization of the gene: it is at least 12 kb long and contains a minimum of six introns, including five in the coding region. Four introns are located at nucleotide positions −33, 286, 533 and 1,142 of the cDNA for the $D_{2(415)}$ receptor, the last one of which has been reported[7]. It is noteworthy that the 87-bp sequence characteristic of the $D_{2(444)}$ receptor is flanked by two other introns, indicating that the two isoforms of the D_2 receptor are products of the same gene, generated by alternative mRNA splicing of the 87-bp exon (Fig. 2). Although all other splice donor and acceptor sequences consist of frequently occurring sequences[8], this is not the case for the splice donor sequence in the intron upstream of the 87-bp exon, which may account for its alternative recognition by the splicing machinery, possibly itself a regulated mechanism. As the 87-bp exon does not contain any splicing consensus sequence, the $D_{2(444)}$ receptor mRNA does not represent an immature mRNA precursor of the shorter form.

Both isoform mRNAs seem to be expressed in all areas of the brain and pituitary in which the D_2 receptor is found[4,9], as indicated by northern analysis using either a probe selective for



Exon	Splice donor	Splice acceptor	Exon
A	CTCAAGgtctctga.......cttcacagGGCAAC		B
B	AGAATGgtaagtgt.......ttccacagGATGCT		C

FIG. 2 Diagram of the D_2 receptor gene showing the alternative splicing sites. Indicated are the positions of introns (lines) and exons (boxes), including the 87-bp exon selectively translated in the case of the $D_{2(444)}$ receptor isoform (shaded box). The corresponding amino acids flanking the exons are also indicated and numbered according to the sequence of the $D_{2(444)}$ receptor isoform. Restriction sites and primers refer to those used to prepare probes for northern blotting and to initiate the PCR as shown in Fig. 3.

METHODS. A rat genomic library (from Dr A. M. Duchemin) constructed in Charon 4A phage from DNA partially digested with *Eco*RI was screened (10^6 phage) using as probe a ^{32}P-labelled nick-translated 1.3-kb *Bam*HI–*Bgl*II fragment of the $D_{2(444)}$ receptor cDNA. Nitrocellulose filters with transferred phage were hybridized for 16 h at 42 °C in 40% formamide, 10% dextran sulphate, 4 × SSC, 8 mM Tris-HCl, pH 7.4, 1 × Denhardt's, 20 μg ml^{-1} yeast tRNA, 20 μg ml^{-1} salmon sperm DNA, 0.1% SDS, using probe at 5×10^5 d.p.m. ml^{-1}. The filters were washed as described in the legend to Fig. 1, except that the last washing was for 30 min at 50 °C. In positive clones, *Eco*RI restriction generated fragments 0.8, 4.5 and 5.5 kb long, all of which hybridized to the *Bam*HI–*Bgl*II probe. The 4.5-kb fragment also hybridized to a probe prepared by ^{32}P-nick translation of the *Rsa*I–*Sac*I fragment of $D_{2(444)}$ receptor cDNA, whereas the other two did not. This fragment was subcloned in M13 mp18 and sequenced using primers located around and within the 87-bp sequence.

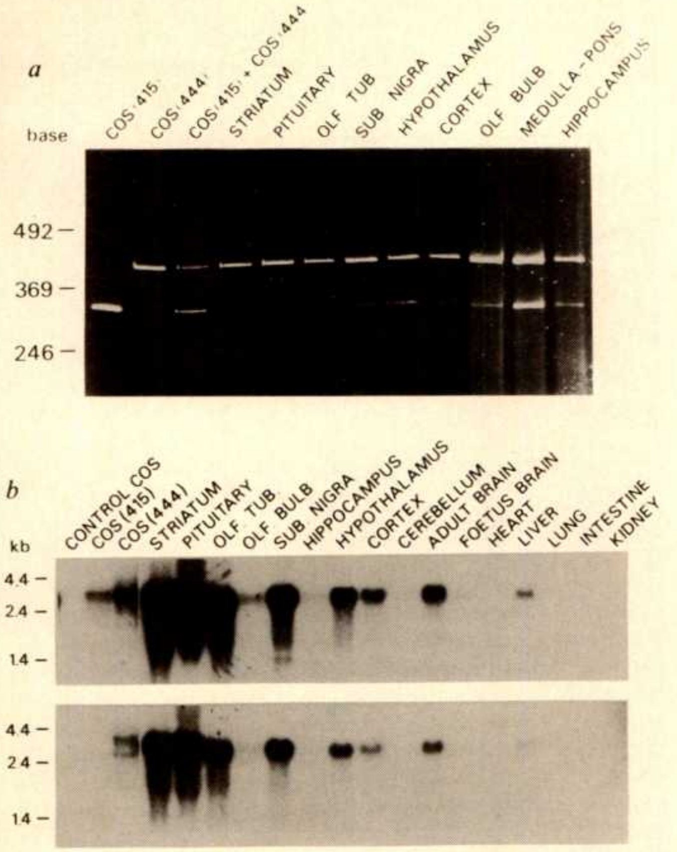


FIG. 3 Characterization of two D_2 dopamine receptor mRNAs. *a*, Northern blot analysis of total RNA from COS-7 cells (10 μg) or poly(A)$^+$ mRNA from rat tissues (5 μg), using as probes either a 241-bp *Sac*I–*Afl*II fragment (top) corresponding to a common sequence of the two isoforms or a 95-bp *Rsa*I–*Sac*I fragment (bottom; different blots from the same samples) corresponding mainly to the alternative exon (Fig. 2). *b*, Electrophoresis of PCR products from RNA using two primers flanking most of the putative third cytoplasmic loop (Fig. 2). The 397- and 310-base products correspond to the $D_{2(444)}$ and $D_{2(415)}$ receptor mRNAs, respectively. Abbreviations: COS(415) and COS(444), COS-7 cells transfected with $D_{2(415)}$ and $D_{2(444)}$ receptor cDNAs respectively; olf. tub., olfactory tubercle; olf. bulb., olfactory bulb; sub. nigra, substantia nigra; cortex, cerebral cortex; foetus brain, brain from gestational day-15 foetus; intestine, duodenum plus ileum.

METHODS. Total RNA was extracted[22] and poly (A)$^+$ mRNA prepared[23] from tissues of male Wistar rats (except for pituitaries, which were from oestradiol-treated females[25]) and COS-7 cells. *Xho*I–*Sal*I fragments of recombinant pGEM-4Z containing $D_{2(415)}$ or $D_{2(444)}$ cDNAs were ligated into the *Xho*I restriction site of the pMAMneo inducible expression vector (Clontech). These constructs (30 μg) were transfected[26] into mouse COS-7 cells (from Dr J. J. Rémi). Transformed cells were selected in 500 μg ml^{-1} G418 and 48 h after transfection, expression was induced by a 12–24 h treatment with 10 μM dexamethasone. For northern blotting, denaturated RNAs were electrophoresed in 1% agarose/formaldehyde gels, blotted onto nitrocellulose paper and baked for 2 h at 80 °C. Membranes were hybridized overnight at 42 °C in 40% formamide, 10 × Denhardt's, 50 mM Tris-HCl, pH 7.4, 4 × SSC, 0.1% sodium pyrophosphate, 1% SDS, 100 μg ml^{-1} salmon sperm DNA and 50 μg ml^{-1} yeast tRNA containing a ^{32}P-labelled nick-translated fragment at 5×10^6 d.p.m. ml^{-1}. For PCR experiments, a single-strand cDNA was synthesized using AMV reverse transcriptase (20 U; Boehringer), primer 2 (5′-GCTTTCTGCGGCTCATCGTCTTAA; Eurogentech) and total RNA (from COS-7 cells containing identical amounts of specific mRNA, as assessed by northern blotting) or 1–6 μg poly(A)$^+$ mRNA (rat tissues). This template was amplified[12], using primer 1 (5′-TTCAGAGCCAACCTGAAGACACCA) and primer 2 (80 nM each) for 35 cycles (92 °C, 54 °C and 72 °C, for 1 min each), with 2.5 U *Taq* DNA polymerase (Perkin Elmer Cetus); 10–20% of PCR products were run on 6% polyacrylamide-urea gel which was stained in ethidium bromide and scanned.

the $D_{2(444)}$ receptor mRNA, or a probe hybridizing to both mRNAs (Fig. 3). In all these tissues, the first probe generated a signal, indicating unambiguously that the $D_{2(444)}$ receptor mRNA was expressed, and its intensity grossly paralleled the relative abundance of D_2 receptors[9]. Interestingly, a faint but distinct signal was also detected with mRNAs from liver, a tissue in which D_2 receptor may be expressed prenatally[10]. In all these tissues, the second probe also generated a signal of apparently parallel relative intensity, but we could not establish from it whether the $D_{2(415)}$ receptor mRNA was also expressed, or its relative abundance. But, using the polymerase chain reaction (PCR)[11,12], we clearly identified the two mRNA isoforms in brain and pituitary from two bands of the predicted sizes (Fig. 3) that hybridized with the *Sac*I–*Afl*II probe (data not shown). The $D_{2(415)}$ receptor mRNA seems to be the least abundant in all regions tested, representing 2% in pituitary, 12% in olfactory

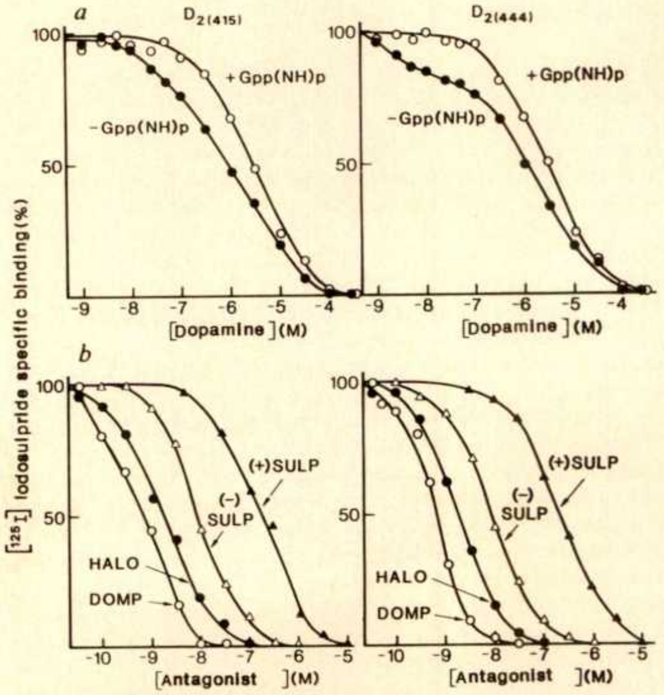


FIG. 4 [^{125}I]iodosulpride binding to the two D_2 receptor isoforms in transfected COS-7 cells. Specific binding of 0.4 nM [^{125}I]iodosulpride (about 2,000 Ci mmol^{-1}; Amersham) represented 100–150 and 150–200 fmol per mg protein in membranes of COS(415) and COS(444) cells respectively. *a*, Effect of Gpp(NH)p, a guanyl nucleotide, on the dopamine inhibition of [^{125}I]iodosulpride binding to $D_{2(415)}$ and $D_{2(444)}$ receptors. In the absence of Gpp(NH)p, dopamine inhibited the specific [^{125}I]iodosulpride binding in a biphasic manner, giving the following IC_{50} values and relative proportions of sites (in parentheses); 120 ± 40 nM (46%) and 6,500 ± 2,900 nM with the $D_{2(415)}$ receptor; 4.7 ± 2.8 nM (22%) and 1,900 ± 420 nM with the $D_{2(444)}$ receptor. In the presence of 0.1 mM Gpp(NH)p, only one site could be detected with IC_{50} values of 2,290 ± 300 nM and 2,490 ± 260 nM for the $D_{2(415)}$ and $D_{2(444)}$ receptors respectively. Means from 3 separate experiments. *b*, Inhibition of specific [^{125}I]iodosulpride binding by various dopaminergic antagonists gave similar IC_{50} values (nM) for the $D_{2(415)}$ and $D_{2(444)}$ receptor isoforms respectively: 0.64 ± 0.10 and 0.61 ± 0.06 with domperidone (DOMP); 1.3 ± 0.1 and 1.3 ± 0.2 with haloperidol (HAL); 9.1 ± 0.5 and 7.9 ± 0.3 with (−)sulpiride ((−)SULP); 190 ± 30 and 170 ± 20 with (+)sulpiride ((+)SULP). Values are means from 2–4 separate experiments.

METHODS. COS-7 cells were scrapped, collected by centrifugation at 1,500*g* for 5 min and washed in 15 mM Tris-HCl buffer, pH 7.4, containing 5 mM $MgCl_2$. After cell disruption, membranes were isolated by centrifugation (twice at 25,000*g* for 10 min) in the same buffer. Binding was assayed in 50 mM Tris-HCl pH 7.4 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 5.6 mM ascorbic acid and 0.1 mM 8-hydroxyquinoleine[9]. Non-specific binding measured in the presence of 1 μM (−)sulpiride typically represented 20–25% of total binding. Curves were analysed by computerized nonlinear regression using a one- or a two-site model[25].

bulb, 20–25% in striatum, olfactory tubercle, substantia nigra, cortex and hippocampus, 40–45% in hypothalamus and pons-medulla. This indicates that the relative level of expression of the two isoforms is tissue-specific.

When expressed in COS-7 cells, which normally lack D_2 receptors, the two receptor isoforms displayed similar pharmacology when studied using $[^{125}I]$iodosulpride, a highly selective D_2 receptor ligand[9] (Fig. 4). This agrees with the idea, derived from site-directed mutagenesis and chimaera construction, that the ligand-binding domain for this family of receptors resides in the transmembrane segments[13,14], which are identical in the two isoforms. It should be emphasized that neither the $D_{2(415)}$ nor the $D_{2(444)}$ receptor display the high affinity for benzamide derivatives which is characteristic of a putative D_2 receptor subtype[5,6]. Both isoforms are apparently coupled to a G protein or proteins in the COS-7 cells, as judged from the modulation of dopamine affinity by a guanyl nucleotide (Fig. 4); this confirms that the absence of this feature in the $D_{2(415)}$ receptor when it is expressed in L cells results from the absence of a suitable G protein, presumably a G_i protein, in the recipient cell[7].

Hence a sizeable deletion in the third cytoplasmic loop, thought to be the domain through which these receptors couple to G proteins, does not prevent coupling; this is consistent with results from deletion mutagenesis[13] and limited trypsinization in β-adrenergic receptors[15]. The significant variation in size and sequence of this loop among receptors of this family may reflect their selective interactions with members of the G-protein family, whose diversity probably results both from multiple genes and alternative splicing[16]. The slightly different pattern of Gpp(NH)p modulation observed with the two receptor isoforms in COS-7 cells (Fig. 4) could reflect either their interaction with different G proteins, or a different interaction with the same G protein.

For neurons, which are long-lived cells that have lost their ability to replicate DNA, alternative splicing is a reversible post-transcriptional mechanism that enables different proteins to be rapidly generated in response to environmental stimuli[17]. The selective tissue expression of the D_2 receptor isoforms by this process reveals a new paradigm in the generation of diversity among members of an already large family and raises questions about the underlying mechanisms and functional implications of this process and their disturbance. For instance, an increased number of D_2 receptors have been detected in the brains of schizophrenic patients and/or following their long-term treatments with antipsychotics[18,19]; it will be interesting to determine which isoforms are involved and whether these changes have aetiological or therapeutic implications. □

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1. Kebabian, J. W. & Calne, D. B. *Nature* **277,** 93–96 (1979).
2. Carlsson, A. & Lindqvist, M. *Acta Pharmac. Toxicol.* **20,** 140–144 (1963).
3. Creese, I., Burt, D. R. & Snyder, S. H. *Science* **192,** 481–483 (1976).
4. Seeman, P. *Pharmacol. Rev.* **32,** 229–313 (1980).
5. Sokoloff, P., Martres, M. P. & Schwartz, J. C. *Nature* **288,** 283–286 (1980).
6. Schwartz, J. C. *et al.* in *Catecholamines: Neuropharmacology and Central Nervous System. Theoretical Aspects* (eds Usdin, E., Carlsson, A., Dahlström, A. & Engel, J.) 59–72 (Liss, New York, 1984).
7. Bunzow, J. R. *et al. Nature* **336,** 783–787 (1988).
8. Shapiro, M. B. & Senapathy, P. *Nucleic Acids Res.* **15,** 7155–7174 (1987).
9. Martres, M. P., Bouthenet, M. L., Salès, N., Sokoloff, P. & Schwartz, J. C. *Science* **228,** 752–755 (1985).
10. Salès, N., Martres, M. P., Bouthenet, M. L. & Schwartz, J. C. *Neuroscience* **28,** 673–700 (1989).
11. Saiki, R. K. *et al. Science* **239,** 487–491 (1988).
12. Doherty, P. J., Huesca-Contreras, M., Dosch, H. M. & Pan, S. *Analyt. Biochem.* **177,** 7–10 (1989).
13. Dixon, R. A. F. *et al. Nature* **326,** 73–77 (1987).
14. Kubo T. *et al. FEBS Lett.* **241,** 119–125 (1988).
15. Rubenstein, R. C., Wong, S. K. F. & Ross, E. M. *J. biol. Chem.* **262,** 16655–16662 (1987).
16. Bray, P. *et al. Proc. natn. Acad. Sci. U.S.A.* **83,** 8893–8897 (1986).
17. Andreadis, A., Gallego, M. E. & Nadal-Ginard, B. *A. Rev. cell. Biol.* **3,** 207–242 (1987).
18. Owen, F. *et al. Lancet* **ii,** 223–226 (1978).
19. Seeman, P. *et al. Science* **225,** 728–731 (1984).
20. Huynh, T. V., Young, R. A. & Davis, R. W. in *DNA Cloning. A Practical Approach* Vol.1 (ed. Glover, D. M.) 49–78 (IRL, Oxford, 1985).
21. Gubler, U. & Hoffman, B. J. *Gene* **25,** 263–269 (1983).
22. Chirgwin, J. J., Przbyla, A. E., MacDonald, R. J. & Rutter, W. J. *Biochemistry* **18,** 5294–5299 (1979).
23. Aviv, H. & Leder, P. *Proc. natn. Acad. Sci. U.S.A.* **69,** 1408–1412 (1972).
24. Sanger, F., Nicklen, S. & Coulson, A. R. *Proc. natn. Acad. Sci. U.S.A.* **74,** 5463–5467 (1977).
25. Sokoloff, P., Martres, M. P., Delandre, M., Redouane, K. & Schwartz, J. C. *Naunyn-Schmiedebergs Archs Pharmak.* **327,** 221–227 (1984).
26. Graham, F. L. & Van der Eb, A. J. *Virology* **52,** 456–467 (1973).

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Multiple D_2 dopamine receptors produced by alternative RNA splicing

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DOPAMINE receptors belong to a large class of neurotransmitter and hormone receptors that are linked to their signal transduction pathways through guanine nucleotide binding regulatory proteins (G proteins). Pharmacological, biochemical and physiological criteria have been used to define two subcategories of dopamine receptors[1] referred to as D_1 and D_2. D_1 receptors activate adenylyl cyclase[2] and are coupled with the G_s regulatory protein[3]. By contrast, activation of D_2 receptors results in various responses including inhibition of adenylyl cyclase[4], inhibition of phosphatidylinositol turnover[5], increase in K^+ channel activity[6] and inhibition of Ca^{2+} mobilization[7]. The G protein(s) linking the D_2 receptors to these responses have not been identified, although D_2 receptors have been shown to both copurify[8,9] and functionally reconstitute[10,11] with both G_i and G_o related proteins[3]. The diversity of responses elicited by D_2-receptor activation could reflect the existence of multiple D_2 receptor subtypes, the identification of which is facilitated by the recent cloning of a complementary DNA encoding a rat D_2 receptor[12]. This receptor exhibits considerable amino-acid homology with other members of the G protein-coupled receptor superfamily[12,13]. Here we report the identification and cloning of a cDNA encoding an RNA splice variant of the rat D_2 receptor cDNA[12]. This cDNA codes for a receptor isoform which is predominantly expressed in the brain and contains an additional 29 amino acids in the third cytoplasmic loop, a region believed to be involved in G protein coupling.

To isolate cDNAs encoding dopamine receptor subtypes, we initially constructed a λ ZAP II cDNA library using mRNA purified from rat striatum, the region of the brain known to contain the highest levels of both D_1 and D_2 dopamine receptors[1]. This library was screened with a mix of two 36-mer synthetic oligonucleotides, the sequences of which were derived from amino acids 352–363 of the rat D_2 receptor cDNA[12]. This region corresponds to the sixth transmembrane-spanning domain and is known to exhibit very high homology among previously cloned G protein-coupled receptors[13]. From 1×10^6 recombinants, 15 positive clones were isolated. Restriction analysis and partial sequence information indicated that 5 of these clones were related to the rat D_2 receptor cDNA reported previously[12]. One of the clones containing an insert of 2.5 kilobases (kb) was completely sequenced and the nucleotide and deduced amino-acid sequence of this cDNA is shown in Fig. 1. The longest open reading frame in this cDNA codes for a 444 residue protein with a relative molecular mass (M_r) of

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      -146   AGGGACGGCGGCCCCGGACGGCTGCCGGAGGGGCGGCCGTGCGTGGATGCGGCGGGAGCTGGAAGCC

 -79   TCGAGCAGCCGGCGCCTTCTCTGGCCCCGGGCGCCATATGGCTTGAAGAGCCGTGCCACCCAGTGGCCCCACTGCCCCA

   1   ATG GAT CCA CTG AAC CTG TCC TGG TAC GAT GAC GAT CTG GAG AGG CAG AAC TGG AGC CGG
       Met Asp Pro Leu Asn Leu Ser Trp Tyr Asp Asp Asp Leu Glu Arg Gln Asn Trp Ser Arg 20

  61   CCC TTC AAT GGG TCA GAA GGG AAG GCA GAC AGG CCC CAC TAC AAC TAC TAT GCC ATG CTG
       Pro Phe Asn Gly Ser Glu Gly Lys Ala Asp Arg Pro His Tyr Asn Tyr Tyr Ala Met Leu 40

 121   CTC ACC CTC CTC ATC TTT ATC ATC GTC TTT GGC AAT GTG CTG GTG TGC ATG GCT GTA TCC
       Leu Thr Leu Leu Ile Phe Ile Ile Val Phe Gly Asn Val Leu Val Cys Met Ala Val Ser 60

 181   CGA GAG AAG GCT TTG CAG ACC ACC ACC AAC TAC TTG ATA GTC AGC CTT GCT GTG GCT GAT
       Arg Glu Lys Ala Leu Gln Thr Thr Thr Asn Tyr Leu Ile Val Ser Leu Ala Val Ala Asp 80

 241   CTT CTG GTG GCC ACA CTG GTA ATG CCG TGG GTT GTC TAC CTG GAG GTG GTG GGT GAG TGG
       Leu Leu Val Ala Thr Leu Val Met Pro Trp Val Val Tyr Leu Glu Val Val Gly Glu Trp 100

 301   AAA TTC AGC AGG ATT CAC TGT GAC ATC TTT GTC ACT CTG GAT GTC ATG ATG TGC ACA GCA
       Lys Phe Ser Arg Ile His Cys Asp Ile Phe Val Thr Leu Asp Val Met Met Cys Thr Ala 120

 361   AGC ATC CTG AAC CTG TGT GCC ATC AGC ATT GAC AGG TAC ACA GCT GTG GCA ATG CCC ATG
       Ser Ile Leu Asn Leu Cys Ala Ile Ser Ile Asp Arg Tyr Thr Ala Val Ala Met Pro Met 140

 421   CTG TAT AAC ACA CGC TAC AGC TCC AAG CGC CGA GTT ACT GTC ATG ATT GCC ATT GTC TGG
       Leu Tyr Asn Thr Arg Tyr Ser Ser Lys Arg Arg Val Thr Val Met Ile Ala Ile Val Trp 160

 481   GTC CTG TCC TTC ACC ATC TCC TGC CCA CTG CTC TTC GGA CTC AAC AAT ACA GAC CAG AAT
       Val Leu Ser Phe Thr Ile Ser Cys Pro Leu Leu Phe Gly Leu Asn Asn Thr Asp Gln Asn 180

 541   GAG TGT ATC ATT GCC AAC CCT GCC TTT GTG GTC TAC TCC TCC ATT GTC TCA TTC TAC GTG
       Glu Cys Ile Ile Ala Asn Pro Ala Phe Val Val Tyr Ser Ser Ile Val Ser Phe Tyr Val 200

 601   CCC TTC ATC GTC ACT CTG CTG GTC TAT ATC AAA ATC TAC ATC GTC CTC CGG AAG CGC CGG
       Pro Phe Ile Val Thr Leu Leu Val Tyr Ile Lys Ile Tyr Ile Val Leu Arg Lys Arg Arg 220

 661   AAG CGG GTC AAC ACC AAG CGC AGC AGT CGA GCT TTC AGA GCC AAC CTG AAG ACA CCA CTC
       Lys Arg Val Asn Thr Lys Arg Ser Ser Arg Ala Phe Arg Ala Asn Leu Lys Thr Pro Leu 240

 721   AAG GGC AAC TGT ACC CAC CCT GAG GAC ATG AAA CTC TGC ACC GTT ATC ATG AAG TCT AAT
       Lys Gly Asn Cys Thr His Pro Glu Asp Met Lys Leu Cys Thr Val Ile Met Lys Ser Asn 260

 781   GGG AGT TTC CCA GTG AAC AGG CGG AGA ATG GAT GCT GCC CGC CGA GCT CAG GAG CTG GAA
       Gly Ser Phe Pro Val Asn Arg Arg Arg Met Asp Ala Ala Arg Arg Ala Gln Glu Leu Glu 280

 841   ATG GAG ATG CTG TCA AGC ACC AGC CCC CCA GAG AGG ACC CGG TAT AGC CCC ATC CCT CCC
       Met Glu Met Leu Ser Ser Thr Ser Pro Pro Glu Arg Thr Arg Tyr Ser Pro Ile Pro Pro 300

 901   AGT CAC CAC CAG CTC ACT CTC CCT GAT CCA TCC CAC CAC GGC CTA CAT AGC AAC CCT GAC
       Ser His His Gln Leu Thr Leu Pro Asp Pro Ser His His Gly Leu His Ser Asn Pro Asp 320

 961   AGT CCT GCC AAA CCA GAG AAG AAT GGG CAC GCC AAG ATT GTC AAT CCC AGG ATT GCC AAG
       Ser Pro Ala Lys Pro Glu Lys Asn Gly His Ala Lys Ile Val Asn Pro Arg Ile Ala Lys 340

1021   TTC TTT GAG ATC CAG ACC ATG CCC AAT GGC AAA ACC CGG ACC TCC CTT AAG ACG ATG AGC
       Phe Phe Glu Ile Gln Thr Met Pro Asn Gly Lys Thr Arg Thr Ser Leu Lys Thr Met Ser 360

1081   CGC AGA AAG CTC TCC CAG CAG AAG GAG AAG AAA GCC ACT CAG ATG CTT GCC ATT GTT CTC
       Arg Arg Lys Leu Ser Gln Gln Lys Glu Lys Lys Ala Thr Gln Met Leu Ala Ile Val Leu 380

1141   GGT GTG TTC ATC ATC TGC TGG CTG CCC TTC TTC ATC ACG CAC ATC CTG AAT ATA CAC TGT
       Gly Val Phe Ile Ile Cys Trp Leu Pro Phe Phe Ile Thr His Ile Leu Asn Ile His Cys 400

1201   GAT TGC AAC ATC CCA CCA GTC CTC TAC AGC GCC TTC ACA TGG CTG GGC TAT GTC AAC AGT
       Asp Cys Asn Ile Pro Pro Val Leu Tyr Ser Ala Phe Thr Trp Leu Gly Tyr Val Asn Ser 420

1261   GCC GTC AAC CCC ATC ATC TAC ACC ACC TTC AAC ATC GAG TTC CGC AAG GCC TTC ATG AAG
       Ala Val Asn Pro Ile Ile Tyr Thr Thr Phe Asn Ile Glu Phe Arg Lys Ala Phe Met Lys 440

1321   ATC TTG CAC TGC TGAGTCTGCCCCTTGCCTGCACAGCAGCTGCTTCCCACCTCCCTGCCTATGCAGGCCAGACCTCAT
       Ile Leu His Cys                                                                 444

1399   CCCTGCAAGCTGTGGGCAGAAAGGCCCAGATGGACTTGGCCTTCTCTCGACCCTGCAGGCCCTGCAGTGTTAGCTTGGCTCG

1481   ATGCCCCTCTCTGCCCACACACCCTCATCCTGCCAGGGTAGGGCCAGGGAGACTGGTATCTTACCAGCTCTGGGGTTGGACC

1563   CATGGCTCAGGGCAGCTCACAGAGTGCCCCTCTCATATCCAGACCCTGTCTCCTTGGCACCAAAGATGCAGCGGCCTTCCTT

1645   GACCTTCCTCTTGGGCACAGAAACTAGCTCAGTGGTCGAGCACACCCTGATCGCTGGCTTGGCCTGGCCCTTGCTTGCCTGT

1727   GCCAGATCAGGTGGTGGGAGGGAGCAACAGTTCTTACTTTATAGGAACCACATAGGAAAGCAGGGAACACGCCAAGTCCTCC

1809   AGGCAACATCAGTGTCAGGAGACACACATAAACACCAGGTAGCTCCATGGACCCCAGAGAAACTGAGGCTGAAAAATCTGTT

1891   TTCCACTCCAACTCTAGTGTGAGTCCCTACTTTTCATAGCCATGGGTATTACTATGTCCTACCTTGTTATAGTATCCCATGG

1973   GGTTTCTGTACCCTTTGGGGGAAAACAACTCTAATCCTCAAGGGCCCCAAGAGAATCTGTAAGGAGAAAAATAGGCTGATCT

2055   CCCTCTACTCTCCAATCCACTCCACCACTTCTTGATATACCTTGGATGTATCCATTCCTCACAGCAAATGCTGGCCAGTCAG

2137   GCCTTGGACCAGTGTTGGAGTTGAAGCTGGATGTGGTAACTTGGGGCTCTTTGGGGCTGGGGGGTTGTTAACATCGTCTCT

2219   CTTCCATATCTCTTCCTTCCCAGTGCCTCTGCCTTAGAAGAGGCTGTGGATGGGGTGCTGGGACTGCTGATACCATTGGGCC

2301   TGGCCCTGAATGAGGAGGGGAAGCTGCAGTTTGGAGGGTTCTGGGATCCAACTCTGTAACATCACTATACCTGCACCAAAAC

2383   TAATAAAACCTTGACAAGAGTCAAAAAAAAAAA
                                 2404
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FIG. 1 Nucleotide and deduced amino-acid sequence of the D_2 receptor cDNA clone. The 87-bp/29 amino-acid insertion sequence is indicated by underlining. The nucleotide sequence is numbered from the initiator Met and indicated at the left of each line. The amino-acid numbers are indicated at the right of each line. The single base differences in the 3' untranslated sequence are indicated by dots.

METHODS. Poly(A)$^+$ RNA was isolated from rat striatal tissue using standard procedures[22] and used to construct a cDNA library in the λ ZAP II vector (Stratagene). The resulting library contained 6.1×10^6 independent recombinants. 1×10^6 recombinants from the unamplified library were screened using the oligonucleotide 5'-GATGAAGAAGGGCAGCCAGCAGATGATGAACACA(G)CC-3' radiolabelled using [γ-^{32}P]ATP and T4 polynucleotide kinase. Duplicate nitrocellulose filters were hybridized in 0.3 M NaCl/0.03 M sodium citrate (2×SSC), 0.02 M Na_2HPO_4, 0.1% SDS, 0.2 mg ml^{-1} salmon sperm DNA, and 4×10^6 d.p.m. ml^{-1} ^{32}P-labelled probe for 18 h at 42 °C. High stringency washing of the filters was performed with 0.5×SSC and 0.1% SDS at 60 °C before autoradiography. Phage λ found to hybridize to the probe were subsequently plaque-purified. *In vivo* excision and rescue of the nested pBluescript plasmids from the λ ZAP II clones were performed using helper phage according to the Stratagene protocol. Nucleotide sequence analysis was performed using the Sanger dideoxy nucleotide chain termination method on denatured doubled-stranded plasmid templates with Sequenase (US Biochemical Corp.). Primers were synthetic oligonucleotides which were either vector-specific or derived from previous sequence information. In some cases a series of nested deletion mutants were constructed using the *Exo* III/Mung Bean nuclease procedure (Stratagene) before DNA sequencing.

50,887. The nucleotide and amino-acid sequence within this coding region is identical to the rat D_2 receptor cDNA previously reported with the notable exception of an additional 87 base pair (bp) sequence coding for a 29 amino-acid insertion between residues 241 and 242 (ref. 12). This is located within the predicted third cytoplasmic loop ~30 residues from the C terminus of the fifth transmembrane-spanning domain. In addition to this insertion sequence, and a slightly extended 5' untranslated sequence, we also noted five base differences within the 3' untranslated region compared with the previously published sequence[12] (Fig. 1). Subsequent sequence analysis indicated that all five of the D_2 receptor-related cDNAs initially isolated from this library contained the identical 87-bp insertion sequence. The nucleotide sequences delineating the boundaries of this insertion sequence correspond with consensus exon sequences for RNA splice junctions[14], indicating that this cDNA results from alternative RNA splicing. The alternative hypothesis, that the different cDNAs are derived from separate genes, is unlikely given the observation that an oligonucleotide probe corresponding to an identical sequence in the two cDNAs (Fig. 2*a*) hybridizes to single DNA fragments on Southern blots of rat genomic DNA cut with a number of different restriction enzymes (data not shown).

To confirm the D_2-subtype identity of this longer cDNA clone and to determine if the 29 amino-acid insertion sequence results in a major alteration in the ligand binding properties of the D_2 receptor, the cDNA was inserted into the SV40 promoter-driven vector, pEUK-C1 (ref. 15), for expression in eukaryotic cells. The resulting plasmid (pEUK-D2L) was used to transiently transfect COS-7 cells by the calcium phosphate precipitation technique[16]. After 3 days, membranes were prepared and the binding of the D_2 dopaminergic antagonist [^{3}H]methylspiperone was examined[17]. In three separate transfection experiments, [^{3}H]methylspiperone bound to the membranes in a saturable fashion with high specific activity ($\sim$1 pmol mg^{-1} protein) and an affinity (62.1 ± 2.1 pM) in good agreement with that found in the rat striatum[1]. No specific binding activity was detected in COS-7 cells that had not been transfected with pEUK-D2L or transfected with the pEUK-Cl vector alone. The ability of a variety of dopaminergic ligands to compete for specific [^{3}H]methylspiperone binding to transfected COS-7 cell membranes was also investigated in these experiments. The high affinity D_2-selective antagonist spiperone (36 ± 3.8 pM) is the most potent agent, followed by the nonselective dopaminergic antagonist (+)butaclamol (0.52 ± 0.01 nM), which is more than four orders of magnitude more potent than its inactive isomer, (−)butaclamol ($>10\ \mu$M). The D_2-selective antagonist (−)sulpiride (7.9 ± 0.42 nM) also exhibits high affinity whereas the D_1-selective antagonist SCH-23390 ($0.41 \pm 0.047\ \mu$M) does not. The Hill coefficients for these antagonist competition curves do not differ significantly from unity. Dopamine is also able to inhibit completely [^{3}H]methylspiperone binding ($K_i = 0.71 \pm 0.012\ \mu$M), although the competition curve is homogeneous (Hill coefficient $\sim$1) and not significantly affected by guanine nucleotides indicating the absence of appropriate G-protein coupling[18] in the COS-7 cells. This rank order of potency as well as the absolute affinities (K_i) of the antagonists agree with those demonstrated for brain D_2 receptors[1], as well as those for the D_2 receptor encoded by the previously cloned cDNA[12]. These preliminary experiments thus indicate that the insertion sequence does not appear to affect the basic ligand recognition properties of the D_2 receptor.

To verify the expression of the D_2 receptor variant containing the insertion sequence and determine the relative proportions of the two receptor isoforms, we subjected various rat tissues to northern blot analysis using an oligonucleotide probe to a consensus region (Fig. 2*a*) as well as an insert sequence-specific

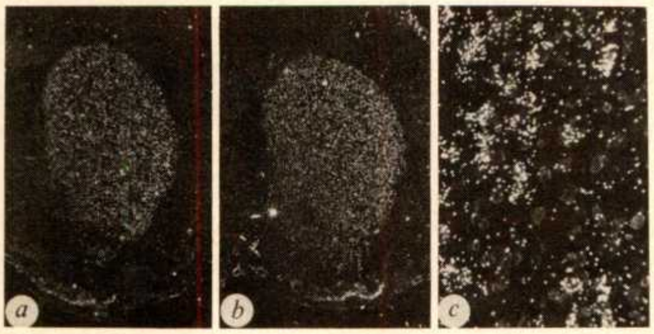

FIG. 2 Northern blot analysis of D_2 receptor transcripts in brain and other rat tissues. Each lane contained 2 μg poly(A)$^+$ RNA. Lanes: 1, total brain; 2, cerebellum; 3, cortex; 4, hippocampus; 5, olfactory bulb; 6, mesencephalon; 7, retina; 8, kidney; 9, striatum; 10, pituitary. The gel locations of the RNA size markers (kb) are indicated. *a*, Blots hybridized with an oligonucleotide derived from amino acids 10–25 (Fig. 1): 5′-TGACCCATTGAAGGGCC-GGCTCCAGTTCTGCCTCTCCAGATCGTCATC-3′. *b*, Hybridization performed with an insert sequence oligonucleotide derived from amino acids 242–257 (Fig. 1); 5′-CATGATAACGGTGCAGAGTTTCATGTCCTCAGGGTGGGTACAGTTG-CC-3′. This experiment was performed twice with similar results.

METHODS. Poly(A)$^+$ RNA was isolated from rat tissues using standard procedures[22] which included 2–3 passages over oligo(dT) cellulose. This procedure resulted in mRNA of high purity nearly devoid of 28S and 18S rRNA. After electrophoresis on 1% agarose plus 0.66 M formaldehyde gels and blotting, the filters were prehybridized in 4 × SSPE, 5 × Denhardt's solution, 50% formamide, 250 μg ml^{-1} yeast tRNA, 500 μg ml^{-1} sheared salmon sperm DNA, and 0.1% SDS for 16 h at 37 °C. The filter blots were then hybridized in the same solution for 18 h at 37 °C with 2×10^6 d.p.m. ml^{-1} oligonucleotide probe radiolabelled with [α-^{32}P]ATP and terminal deoxytransferase. The blots were washed in 1 × SSPE and 0.1% SDS for 20 min four times at 56 °C and twice at room temperature before autoradiography.

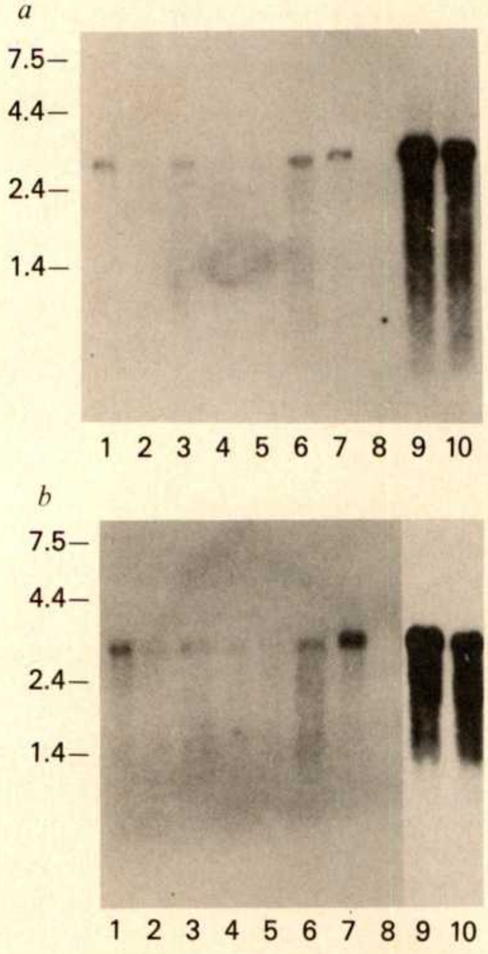

FIG. 3 Darkfield photomicrographs (silver grains appear white) of *in situ* hybridization histochemical (ISHH) localization of D_2 receptor mRNA in a coronal section of rat brain which includes the striatum. *a*, ISHH labelling using the ^{35}S-labelled oligonucleotide from Fig. 2*a*. *b*, ISHH labelling using the ^{35}S-labelled oligonucleotide from Fig. 2*b*. In both sections the labelling of cells is most dense in the striatum and olfactory tubercle. *c*, High power magnification of labelling from *b* showing localization of D_2 receptor mRNA in a subset of medium-sized neurons in the striatum. Approximately half of the medium-sized cells are labelled by this procedure.

METHODS. Coronal sections through the striatum were cut in a cryostat and adhered to glass slides that had been twice coated with gelatin. Sections were fixed in a 4% paraformaldehyde solution in 0.9% saline for 10 min, rinsed and incubated in a fresh solution of 0.25% acetic anhydride in 0.1 M triethanolamine and 0.9% saline (pH 8) for 10 min, dehydrated in ethanol and defatted for 2 × 5 min in chloroform, rehydrated and air-dried. These sections were then hybridized with the ^{35}S-dATP tailed oligonucleotide probes and processed as previously described[23]. Subsequently, the slides were dipped in NTB3 emulsion (diluted 1:1 with water) and exposed for 4–6 weeks, after which they were developed in D-19 developer for 2 min, fixed, rinsed, counterstained with thionin, dehydrated and coverslipped out of xylene.

probe (Fig. 2*b*). As shown in Fig. 2, the tissues expressing the highest levels of the 2.9-kb D_2 receptor mRNA are the striatum and pituitary. The retina shows a moderate abundance of mRNA with low levels being observed in the mesencephalon and cortex and trace quantities detected in the olfactory bulb and hippocampus. Little mRNA was found in the cerebellum and kidney. This tissue distribution corresponds closely to that previously determined for D_2 receptor expression[1]. Of interest, however, is the fact that in all of the tissues examined, the amount of mRNA detected with the two probes is very similar (Fig. 2) and in no instance did the consensus probe detect greater quantities of mRNA.

To investigate further the relative distributions of the two mRNAs encoding the D_2 receptor isoforms we performed *in situ* hybridization analysis in the rat forebrain with the two oligonucleotide probes used in Fig. 2. Figure 3 shows darkfield photomicrographs of *in situ* hybridization histochemical localization of D_2 receptor mRNA in a coronal section of rat brain which includes the striatum. As can be seen, identical patterns of labelling were obtained using both the consensus region probe and the insert sequence probe (Fig. 3*a*, *b*). The highest labelling occurred in the striatal neurons where $\sim$50% of the medium sized neurons were labelled (Fig. 3*c*). Larger sized neurons in the striatum also exhibited labelling (data not shown).

It is interesting that in Figs 2 and 3 there did not appear to be any difference in the levels of mRNA detected using the two oligonucleotide probes. If any tissue or brain area expressed mRNA containing the insertion sequence at a level equal to or less than the one lacking the insertion, then the consensus probe should detect mRNA levels that are at least twofold greater than those seen with the insert probe. These experiments thus indicate that not only is the longer D_2 receptor variant (which we propose designating D_{2L}) expressed in brain and other tissues, but in those areas which have been examined (especially the striatum), it appears to be the major isoform. Exploring this further, we rescreened our unamplified rat striatal cDNA library at high stringency using the full length D_{2L} receptor cDNA as a probe, which resulted in the isolation of $\sim$24 D_2-receptor-positive clones. Partial sequence analysis indicated that only three of these clones correspond to the previously isolated cDNA[12] encoding the shorter D_2 receptor (now designated D_{2S}). This indicates that the D_{2S} receptor is, in fact, expressed in the striatum, albeit in low abundance. Further experiments directed at determining the actual levels of the receptor proteins will be required to confirm this point.

With the exception of the visual opsins, the genes for the G protein-coupled receptor family have, in most instances, demonstrated a lack of introns within their coding sequences[13] thus precluding the generation of receptor diversity through alternative RNA splicing. Recently, however, it has been determined that the serotonin 5HT$_{1C}$ (B.J. Hoffmann, personal communication) and D_2 dopamine[12] receptors are encoded by genes that contain introns. Our data on the rat D_2 receptor now provides an example of G protein-coupled receptor isoforms generated through alternative RNA splicing. These isoforms, which are defined by the presence or absence of a 29-amino-acid sequence within the receptor protein, could arise either through the existence of a 'cassette exon' or through alternative internal acceptor or donor sites within the precursor mRNA[19]. The isolation and sequencing of the rat D_2 receptor gene will be required to distinguish among these possibilities. The location of this optional amino-acid sequence is particularly intriguing as it occurs within the predicted third cytoplasmic loop of the receptor[12]. Recent mutagenesis studies using the β_2-adrenergic catecholamine receptor have indicated that this region is involved in G protein-receptor coupling[20,21]. It is thus tempting to speculate that the two D_2 receptor isoforms are coupled to different G proteins thus resulting in the diversity of responses associated with D_2 receptor activation[4-7]. Further work involving the stable expression of the two D_2 receptor isoforms in cells exhibiting appropriate G protein-linked effector systems will be required to test this hypothesis. □

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1. Eds. Creese, I. & Fraser, C. M. *Receptor Biochemistry and Methodology: Dopamine Receptors* **8,** (Liss, New York, 1987).
2. Kebabian, J. W. *et al. Trends Pharmacol.* **7,** 96–99 (1986).
3. Freissmuth, M. Casey, P. J. & Gilman, A. G. *FASEB J.* **3**, 2125–2132 (1989).
4. Creese, I., Sibley, D. R., Hamblin, M. W. & Leff, S. E. *A. Rev. Neurosci.* **6,** 43–71 (1983).
5. Vallar, L. & Meldolesi, J. *Trends Pharmacol.* **10**, 74–77 (1989).
6. Lacey, M. G., Mercuri, N. B. & North, R. A. *J. Physiol.* **392,** 397–416 (1987).
7. Bigornia, L. *et al. J. Neurochem.* **51**, 999–1006 (1988).
8. Senogles, S. E. *et al. J. biol. Chem.* **262,** 4860–4867 (1987).
9. Elazar, Z., Siegel, G. & Fuchs, S. *EMBO J.* **8**, 2353–2357 (1989).
10. Senogles, S. E., Amlaiky, N., Falardeau, P. & Caron, M. G. *J. biol. Chem.* **263**, 18996–19002 (1988).
11. Ohara, K. *et al. Molec. Pharm.* **33,** 290–296 (1988).
12. Bunzow, J. R. *et al. Nature* **336,** 783–787 (1988).
13. O'Dowd, B. F., Lefkowitz, R. J. & Caron, M. G. *A. Rev. Neurosci.* **12**, 67–83 (1989).
14. Mount, S. M. *Nucleic Acids. Res.* **10,** 459–472 (1982).
15. Templton, D. & Eckhart, W. *J. molec. Cell Biol.* **4,** 817–821 (1984).
16. Chen, C. & Okayama, H., *J. molec. Cell Biol.* **7,** 2745–2752 (1987).
17. Monsma, F. J., Jr., Brassard, D. L. & Sibley, D. R. *Brain Res.* **492,** 214–324 (1989).
18. Sibley, D. R., De Lean, A. & Creese, I. *J. biol. Chem.* **257,** 6351–6361 (1982).
19. Andreadis, A., Gallego, M. E., Nadal-Ginard, B. *A. Rev. Cell Biol.* **3,** 207–242 (1987).
20. Strader, C. D., Sigal, I. S. & Dixon, R. A. F. *FASEB J.* **3,** 1825–1832 (1989).
21. O'Dowd, B. F. *et al. J. biol. Chem.* **263,** 15985–15992 (1988).
22. Okayama, H. *et al. Meth. Enzym.* **154,** 3–28 (1987).
23. Gerfen, C. R. & Young, W. S. *Brain Res.* **460**, 161–167 (1988).

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Mechanism of antigen-driven selection in germinal centres

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THE high affinity of antibodies produced during responses to T-cell-dependent antigens is associated with somatic mutation in the variable region of the immunoglobulin[1-4]. Indirect evidence indicates that: (1) this arises by a process of hypermutation, acting selectively on rearranged immunoglobulin variable-region genes, which is activated in centroblasts within germinal centres; and (2) centrocytes, the progeny of centroblasts, undergo selection on the basis of their ability to receive a positive signal from antigen[5]. We have now performed experiments analysing this selection process, and found that, on culture, centrocytes isolated from human tonsil kill themselves within a few hours by apoptosis[6]. This is not a feature of other tonsillar B cells. Centrocytes can be prevented from entering apoptosis if they are activated both through their receptors for antigen and a surface glycoprotein recognized by CD40 antibodies[7,8].

Immunohistological studies have shown that B cells undergo a marked phenotypic change when they participate in germinal-centre reactions[8]. The lack of surface IgD and CD39 antigen on either centroblasts or centrocytes was used to isolate human germinal-centre B cells by negative selection from suspensions of medium- and low-density tonsillar B cells (Table 1). The phenotype of the resulting CD39-IgD-negative fraction (Table 1) corresponds with that defined for germinal-centre B cells using immunohistology[8]. These cells show good viability at the end of the isolation procedure, carried out at 4 °C. When these cells were cultured at 37 °C they began to show signs of apoptosis (Fig. 1*a*). This process resulted in the loss of $\sim$75% of the cells within 16 h, with no cells surviving 40 h (Table 2). By contrast, apoptosis was not a feature either of the medium-to-low density tonsillar B cells that expressed CD39 or IgD, or both, or of dense tonsillar B cells (Table 2). Similar results were obtained when germinal-centre cells were positively selected for expression of CD38 (Table 2). Apoptosis in the germinal-centre cell-enriched preparation was confirmed by: (1) finding the characteristic DNA fragmentation, caused by endonuclease activity which cuts DNA at intervals of about 180 bases[6], using methods described previously[9]; and (2) identifying the morphological features of apoptosis, which include chromatin condensation with nuclear fragmentation, by both conventional (Fig. 1*a*) and electron microscopy[6].

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Follicular dendritic cells take up antigen, in the form of immune complex, and retain this for extended periods[10,11]. These may provide a means for antigen-driven B-cell selection. A series of experiments were carried out to see if apoptosis in isolated germinal-centre cells could be prevented by exposing the cells to a range of antibodies to human immunoglobulins. None of these prevented apoptosis when added in soluble form to cultures. When polyspecific anti-immunoglobulin was conjugated to sheep red blood cells and added to cultures, however, it inhibited apoptosis (Table 3*a*, Fig. 1*b*). This protective effect was also obtained with anti-IgG, but not with other immunoglobulin class-specific antibodies bound to red cells (Table 3*b*). IgG was the only class of immunoglobulin expressed on a substantial proportion of isolated germinal-centre cells (Table 1).

Germinal-centre B cells were not prevented from entering apoptosis when they were cultured with a range of other agents that stimulate B cells. Among such agents tested were the recombinant cytokines, interleukin (IL)-1, IL-2, IL-3, IL-4, IL-5, IL-6 and IL-7, CD23 antibody, gamma and alpha interferon and supernatant from mixed lymphocyte reaction cultures (data not detailed). These agents were all added in concentration ranges known to be biologically active in control human B-cell cultures. By contrast, the addition of soluble CD40 antibody greatly reduced the number of cells entering apoptosis (Table 3*a*). Although anti-immunoglobulin and CD40 were effective in delaying the onset of apoptosis, neither agent used in isolation was able to sustain viability beyond 2 days of culture. Good survival after 4 days of culture was obtained when the two agents were added in combination (Table 3*a*). The CD40 effect could reflect a microenvironment-dependent signal.

The trigger for apoptosis in cultured germinal-centre cells is unclear. It is unlikely that the cells are killed by cytolytic lymphocytes, because apoptosis continues in the absence of extracellular divalent cations (data not shown). This was demonstrated using techniques described in ref. 12. Calcium, and to a lesser extent Mg^{2+}, are required for lymphocyte-mediated

TABLE 1 Phenotype of tonsil B cells

	B-cell fraction	
Antigen detected	Enriched for germinal-centre cells (sCD39 −ve and sIgD −ve)	Depleted of germinal-centre cells (sCD39 +ve and/or sIgD +ve)
sCD19	85 (79–95)	88 (84–98)
sCD10	74 (65–80)	22 (15–30)
sCD38	86 (81–92)	32 (23–50)
sCD39	0 (0–2)	53 (38–75)
sCD3	0 (0–0)	0 (0–0)
sIgG	47 (28–52)	34 (24–43)
sIgM	3 (1–16)	26 (24–37)
sIgD	0 (0–0)	60 (54–70)
sIgA	0 (0-0)	2 (1–4)
Alpha-NAE	5 (4–5)	3 (3–3)

The phenotype of tonsil B cells either enriched for germinal-centre cells by removal of cells binding CD39 antibody or anti-IgD, or both, or depleted of germinal-centre cells. The values shown are the median value for the percentage of cells positive, based on five experiments, with the range in parentheses. Tonsil B cells were prepared as previously described[24]; all separation procedures were performed at 4 °C. High-density cells which lack the features of germinal-centre B cells were removed by centrifugation through a 60% Percoll gradient. The remaining cells were mixed with sheep red cells coated with CD39 antibody AC-2 (ref. 8) and red cells coated with sheep anti-human IgD. After 30 min, the non-rosetting, germinal-centre cell-enriched fraction was separated from the rosetting fraction using Ficoll-isopaque density gradient centrifugation. The surface (s) phenotype of the cells was determined using the direct antibody rosette technique[25]. The nuclei of the cells were stained with 0.002% acridine orange to identify cells undergoing apoptosis and nonmononuclear cells. These were excluded from the counts. Alpha-napthyl-acetate esterase (alpha-NAE) activity, used to identify macrophages, was revealed as described in ref. 26. The following monoclonal antibodies were used: CD19, an antigen present on all B cells (BU-12; ref. 8); CD10, CALLA, present on germinal-centre B-lineage cells but not other tonsil B cells (AL2; ref. 27); CD38, identifies germinal-centre B-lineage cells and plasma cells, but not other B cells found in the tonsil (OKT10; ref. 28); CD39, an antigen present on most tonsilar B cells, other than germinal-centre B-lineage cells, as well as some macrophages and plasma cells (AC-2; ref. 8); CD3, present on all T cells (OKT3; ref. 29); anti-IgG (8A4; ref. 30); anti-IgM (AF6; ref. 8). Sheep antibodies against human: IgA, IgD and IgM and IgG were used (The Binding Site, Birmingham, UK). Abbreviations: +ve, positive; −ve, negative.

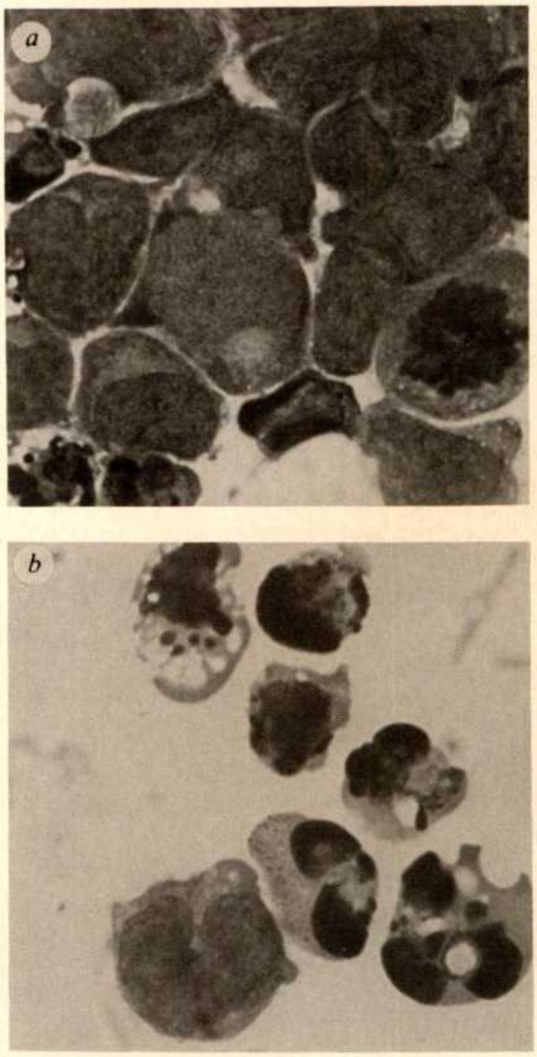

FIG. 1 *a*, Cells from the germinal-centre cell fraction of human tonsil after 6 h culture as described in Table 1. One intact centroblast is seen with six apoptotic cells which show the nuclear condensation and fragmentation which characterize this process[6]. This is a Romanowski-stained cytocentrifuge preparation photographed at a microscope magnification of ×1,000. *b*, Cells from the same germinal-centre fraction depicted in *a*, cultured with sheep red cells coated with polyvalent anti-human immunoglobulin for 16 h as described in Table 1. Staining and photography as in *a*. Blast cells with frequent mitotic figures characterize these preparations. The cells tend to grow in clusters with some apoptotic cells still found at the periphery of these clusters.

TABLE 2 Selective cell death in germinal-centre B cells

Tonsil B-cell fraction	% cells recovered from cultures after: 16 h	40 h	72 h	96 h
High-density resting B cells	96–99	60–81	54–58	30–39
Low- and medium-density CD39 and sIgD −ve	22–26	0–0	0–0	0–0
Low- and medium-density CD39 and/or sIgD +ve	64–73	48–56	41–50	20–30
Low- and medium-density CD38 +ve	38–42	19–21	10–12	2–8
Low- and medium-density CD38 −ve	87–95	68–90	68–90	50–85

The first three tonsil B-cell fractions were prepared as described in Table 1, the CD38 positive (+ve) and CD38 negative (−ve) fractions were prepared in a similar way using CD38-coated sheep red cells. The CD38 +ve fraction contains all germinal-centre B-lineage cells but does include some cells from outside germinal centres (Table 1). Cell fractions were cultured for the periods indicated in flat-bottomed microtitre wells at 37 °C in a moist atmosphere of 5% CO_2 in air in 200 μl of RPMI 1640 medium enriched with 10% fetal bovine serum. The starting cell concentration was 2×10^5 cells per ml. The number of viable cells recovered in two separate experiments from wells after the periods of culture indicated is shown. Cell viability was assessed by trypan-blue exclusion.

cytolysis[12,13]. The macrophage content of the germinal-centre cell fraction (Table 1) is similar to that in the fraction positive for CD39 or IgD, or both. It is difficult to understand why there should be selective macrophage-mediated lysis of the germinal-centre cells and why this should be inhibited by CD40. If the germinal-centre cells are not being killed, they must be killing themselves. The high physiological cell death rate among these cells *in vivo*[14,15], together with the finding that other tonsil B cell populations do not destroy themselves by apoptosis on culture (Table 2), seems to argue against this being an artefact of the culture system.

Affinity maturation of antibodies in responses to T-cell-dependent antigens is mainly attributable to alteration of the structure of the immunoglobulin variable region by somatic mutation[4]. The time of onset of this somatic mutation[16] correlates with the start of the germinal-centre reaction[15,17]. Other features consistent with a link between these processes have been described previously[5]. Mutations can increase, decrease or fail to modify the affinity of antibodies. The experiments reported here indicate how centrocytes with low affinity for antigen can be eliminated. The germinal-centre cells with high affinity are most likely to be those which survive to give rise to memory B cells[18,19].

TABLE 3 Ability of antibodies to prevent cell death

a, Soluble CD40 antibody and anti-human immunoglobulin

Culture additive	% Viable cells (mean ± s.e.m. of 5 expts) recovered after: 16 h	40 h	72 h	96 h
None	20 ± 6	0	0	0
Anti-Ig-coated sheep red cells	76 ± 6	23 ± 4	6 ± 8	0
Soluble CD40*	75 ± 12	39 ± 16	17 ± 4	0
Anti-Ig-coated sheep red cells plus soluble CD40*	86 ± 6	70 ± 12	40 ± 18	40 ± 24

b, Antibodies against different human immunoglobulin classes

Antibody added to coat sheep red blood cells	% Viable cells (mean ± s.e.m. of 5 expts) recovered after 16-h culture
None	20 ± 10
Polyvalent anti-Ig	70 ± 8
anti-IgG	54 ± 8
anti-IgM	24 ± 10
anti-IgD	26 ± 8
anti-IgA	23 ± 3

a, The prevention of cell death in cultures of germinal-centre cells by the addition of soluble CD40 antibody and sheep red cells coated with anti-human immunoglobulin. *b*, The relative ability of antibodies against different human immunoglobulin classes to prevent cell death in isolated germinal-centre cells. The germinal-centre cells used in these experiments were isolated as described in Table 1. They were cultured and their viability assessed as described in Table 2. The anti-immunoglobulins (Ig) used and the method used for their conjugation to sheep red cells is specified in Table 1.

* CD40 antibody G28-5 was added to cultures at a final concentration of 5 μg ml^{-1} (ref. 7).

Defects in the selection system identified in this study could be involved in the pathogenesis of low-grade follicular lymphomas. These lymphomas have a (14; 18) translocation involving apposition of the proto-oncogene *bcl-2* and the immunoglobulin heavy-chain locus[20,21]. Vaux *et al.*[22] showed that expression of *bcl-2* transfected into IL-3-dependent cell lines prevented cell death on withdrawal of IL-3. The introduction of constructs representing the *bcl-2*-immunoglobulin gene fusion into the germ line of mice leads to the accumulation of mature B cells in the adult mice[23]. It is an intriguing possibility that the *bcl-2* gene product may be involved in overriding the apoptotic tendency of germinal-centre cells. □

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1. Gearhart, P. J., Johnson, N. D., Douglas, R. & Hood, L. *Nature* **291,** 29–34 (1981).
2. Bothwell, A. M. *et al. Cell* **24,** 625–637 (1981).
3. Tonegawa, S. *Nature* **302,** 575–581 (1983).
4. Griffiths, G. M., Berek, C., Kaartinen, M. & Milstein, C. *Nature* **312,** 271–275 (1984).
5. MacLennan, I. C. M. & Gray, D. *Immunol. Rev.* **91,** 61–85 (1986).
6. Wyllie, A. H., Morris, R. G., Smith, A. L. & Dunlop, D. *J. Path.* **142,** 67–77 (1982).
7. Clark, E. A. & Ledbetter, J. A. *Proc. natn. Acad. Sci. U.S.A.* **83,** 4494–4498 (1986).
8. Ling, N. R., MacLennan, I. C. M. & Mason, D. Y. in *Leucocyte Typing III* (eds A. J. McMichael *et al.*) 302–335 (Oxford University Press, 1987).
9. Smith, C. A., Williams, G. T., Kingston, R., Jenkinson, E. J. & Owen, J. J. T. *Nature* **337,** 181–184 (1989).
10. Tew, J. G. & Mandel, T. E. *Immunology* **37,** 69–76 (1979).
11. Szakal, A. K., Kosco, M. H. & Tew, J. G. *J. Immun.* **140,** 341–353 (1988).
12. MacLennan, I. C. M., Gotch, F. M. & Golstein, P. *Immunology* **39,** 109–117 (1980).
13. MacLennan, I. C. M. & Golstein, P. *J. Immun.* **121,** 2542–2546 (1978).
14. Fliedner, T. M., Kesse, M., Cronkite, E. P. & Robertson, J. S. *Ann. N.Y. Acad. Sci.* **11,** 578–594 (1964).
15. MacLennan, I. C. M., Liu, Y-J. & Ling, N. R. *Curr. Topics Microbiol. Immunol.* **141,** 138–148 (1988).
16. Berek, C. & Milstein, S. *Immunol. Rev.* **96,** 23–41 (1987).
17. Kraal, G., Hardy, R. R., Gallatin, W. M., Weissman, I. R. & Butcher, E. C. *Eur. J. Immun.* **16,** 829–834 (1986).
18. Klaus, G. G. B., Humphrey, J. H., Kunkle, A. & Dongworth, D. W. *Immunol. Rev.* **53,** 3–28 (1980).
19. Coico, R. F., Bhogal, B. S. & Thorbecke, G. J. *J. Immun.* **131,** 2254–2257 (1983).
20. Tsujimoto, Y., Cossman, J., Jaffe, E. & Croce, C. M. *Science* **228,** 1440–1443 (1985).
21. Graninger, W. B., Seto, M., Boutain, B., Goldman, P. & Korsemyre, S. J. *J. clin. Invest.* **80,** 1512–1515 (1987).
22. Vaux, D. L., Cory, S. & Adams, J. M. *Nature* **335,** 440–442 (1988).
23. McDonnell, T. J. *et al. Cell* **57,** 79–88 (1989).
24. Walker, L. *et al. Immunology* **58,** 533–589 (1986).
25. Ling, N. R., Bishop, S. & Jefferis, R. *J. immunol. Meth.* **15,** 279–289 (1979).
26. Gill, P. G., Waller, X. A. & MacLennan, I. C. M. *Immunology* **33,** 873–880 (1977).
27. Lebacq-Verheyden, A. M. *et al. Int. J. Cancer* **32,** 237–279 (1983).
28. Van Camp, B., Thielmans, C. & Dehou, M. F. *J. clin. Immun.* **2,** 67–74 (1982).
29. Kung, P. C., Goldstein, G., Reinhertz, E. L. & Schlossman, S. F. *Science* **206,** 347–349 (1979).
30. Partridge, L. J., Lowe, J., Hardie, D. L., Ling, N. R. & Jefferis, R. *J. Immun.* **128,** 1–6 (1982).

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Blockage of $\alpha\beta$ T-cell development by TCR $\gamma\delta$ transgenes

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T LYMPHOCYTES recognize antigens by means of T-cell receptors (TCR) composed of $\alpha\beta$ (refs 1–3) or $\gamma\delta$ heterodimers[4–6]. The mechanism governing the development of $\alpha\beta$- and $\gamma\delta$-bearing T cells from a common precursor T cell is so far unknown. It has been proposed that T-cell precursors rearrange their γ- and δ-chain genes first, and $\alpha\beta$ T cells are generated only from those cells that fail to rearrange productively both γ- and δ-chain genes[7,8]. Our recent study on $\gamma\delta$-transgenic mice contradicted this

hypothesis, however, and indicated that repression of γ-chain gene expression mediated by a transcriptional silencer element has a critical role in the generation of $\alpha\beta$ T cells[9]. Here we report that the generation of $\alpha\beta$ T cells is severely blocked in transgenic mice carrying γ- and δ-chain transgenes without the associated silencer, thereby strengthening the validity of the silencer model of T-cell development.

Our recent analysis of $\gamma\delta$-transgenic mice (called KN6 transgenic mice) constructed with relatively long (~40 kilobases (kb) each) $V_4J_1C_1\gamma$ and $V_5DJ_1C\delta$ genomic DNA clones indicated that, in contrast to the Pardoll-Allison model[7,8], introduction of productively rearranged γ- and δ-chain genes into the germline does not prevent the rearrangement of the endogenous α- and β-chain genes and their cell-surface expression[9]. In these transgenic mice, transcription of the γ-chain transgene is repressed in $\alpha\beta$ T cells apparently through a *cis*-acting DNA element (silencer) present in the flanking region(s) of the γ-chain gene. This led us to propose a model of T-cell development in which commitment of a given T-cell precursor to the $\alpha\beta$-lineage depends on the activation of the machinery acting on the silencer[9]. We have now constructed transgenic mice with γ- and δ-chain genes carrying no silencer element so as to perform the crucial test of the silencer model. The model predicts that the transgenes will be transcribed in all T cells in these mice, as opposed to the KN6 transgenic mice, and that their subsequent surface expression will prevent rearrangement and expression of TCR $\alpha\beta$-chain genes, in analogy to the immunoglobulin system[10].

As transgenes we used 15-kb and 21-kb genomic DNA fragments (Fig. 1) containing productively rearranged $V_5J_1C_1\gamma$ and $V_1D_2J_2C\delta$ genes, respectively, known to encode the $\gamma\delta$ TCR expressed on the surface of most early fetal thymocytes[11,12] and dendritic epidermal $\gamma\delta$ T cells (DEC)[11,13]. According to our previous study it is probable that the 15-kb γ-chain gene clone lacks the putative silencer element, because the transcription of a γ-chain transgene containing similar flanking regions is not repressed in $\alpha\beta$ T cells[9]. We analysed the progeny of two transgenic founders (henceforth called DEC transgenic mice).

Cell-surface expression of transgene-encoded TCR was investigated by flow cytometry, using monoclonal antibodies reactive with all $\gamma\delta$ TCR (3A10)[14] or $V_{\gamma5}$-encoded γ-chains only (F536)[11]. Consistent with previous reports, few if any $\gamma\delta$ T cells from nontransgenic adult mice expressed $V_{\gamma5}$-encoded $\gamma\delta$ TCR on their surface (Fig. 2*a*)[11,12]. By contrast, more than 95% of $\gamma\delta$ thymocytes or splenic T cells from the DEC transgenic mice were reactive with the F536 monoclonal antibody (Fig. 2*a*, and data not shown), indicating that, like the KN6 $\gamma\delta$ transgenic mice (Fig. 2*a* and ref. 9), the transgenes dominated the $\gamma\delta$ T cells for the expression of their TCR. The proportion of $\gamma\delta$ T cells in the thymus from DEC transgenic mice (25-75%) was dramatically increased in comparison with nontransgenic mice (0.5%) or KN6 transgenic mice (4.5%) (Fig. 2*a*). In addition, unlike KN6 transgenic mice, the thymus size was severely reduced in DEC transgenic mice (data not shown), suggesting that the generation of the main thymocyte subpopulation, that is, $\alpha\beta\text{TCR}^+$ cells, was strongly hampered in these animals.

This supposition was confirmed by immunofluorescence analysis using the anti-$\alpha\beta$TCR monoclonal antibody H57-597[15]; less than 3% of thymocytes from 7-day-old DEC transgenic mice were $\alpha\beta\text{TCR}^+$, whereas most (>60%) thymocytes from the age-matched control mice or KN6 transgenic mice carried $\alpha\beta$ TCR (Fig. 2*b*). The proportion of $\alpha\beta\text{TCR}^+$ thymocytes increased with age to reach 50% in 10-week-old mice (Fig. 2*b*). But because the total number of thymocytes was greatly reduced in the DEC transgenic mice, the absolute number of $\alpha\beta$ chain-bearing thymocytes never reached more than 5% of the level observed in nontransgenic mice (data not shown).

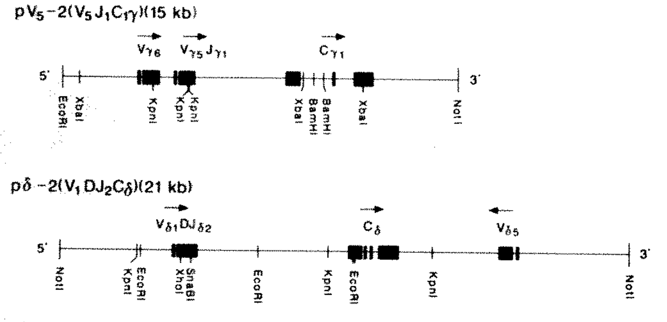

FIG. 1 Restriction maps of γ- and δ-chain genomic DNA clones used for the construction of transgenic mice. Filled boxes designate exons or gene segments; arrows above indicate transcriptional orientation.

METHODS. High-relative molecular mass DNA was isolated from a $\gamma\delta$ T-cell hybridoma KI-129[12] as previously described[22] and was used to construct a cosmid library using the plasmid pWe15 vector[23]. The library was screened with ^{32}P-labelled $V_{\gamma5}$, C_γ, $V_{\delta1}$ or C_δ probes for the cloning of the γ- and δ-chain genes. The C_γ and C_δ probes have been previously described[24,25]. Screening led to the isolation of clones pV5-2 and *p*δ-2, containing the entire γ- and δ-chain genes expressed by the KI-129 hybridoma. The junctional sequences of clones pV5-2 and *p*δ-2 were identical to those published[13,26]. The $V_{\gamma5}$ gene was assigned according to ref. 27. The $\gamma\delta$-transgenic mice were generated after injection into fertilized mouse eggs of purified pV5-2 and *p*δ-2 DNA as previously described[5,28].

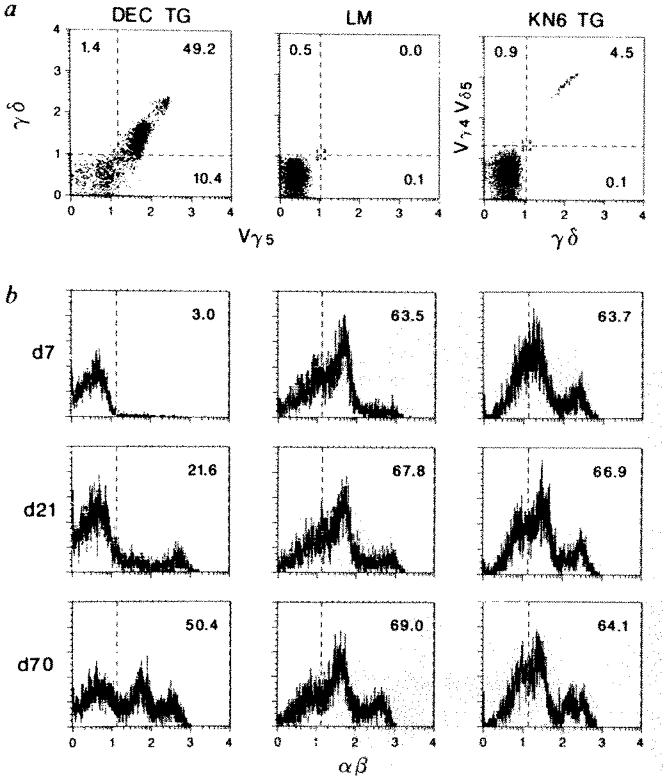

FIG. 2 Surface expression of $\gamma\delta$ and $\alpha\beta$ TCR by thymocytes from DEC transgenic, KN6 transgenic and nontransgenic mice. *a*, Thymocytes from 70-day-old DEC transgenic (left) and nontransgenic (middle) litter-mate mice were stained with anti-$V_{\gamma5}$ monoclonal antibody F536 followed by fluorescein isothiocyanate (FITC)-coupled goat anti-hamster IgG and with biotinylated anti-$\gamma\delta$-chain monoclonal antibody 3A10 followed by phycoerythrin-streptavidin. Shown are the dot-plot histograms (F536, horizontal axis; 3A10, vertical axis) obtained after flow cytometric analysis using a FACSCAN (Becton-Dickinson) and the percentage of cells in each quadrant. Fluorescence intensity is expressed in $\log_{10}$ units. For comparison is shown the dot-plot histogram (3A10, horizontal axis; 8D6 (anti-$V_{\gamma4}V_{\delta5}$)[14], vertical axis) of thymocytes from age-matched KN6 transgenic mice[9] (right). *b*, Thymocytes from 7-, 21- and 70-day-old DEC transgenic (left), nontransgenic (centre) and KN6 transgenic (right) mice were stained with biotinylated anti-$\alpha\beta$-chain monoclonal antibody H57-597 followed by phycoerythrin-streptavidin. The percentages of positive cells are indicated to the right of each histogram. Fluorescence intensity is expressed in $\log_{10}$ units.

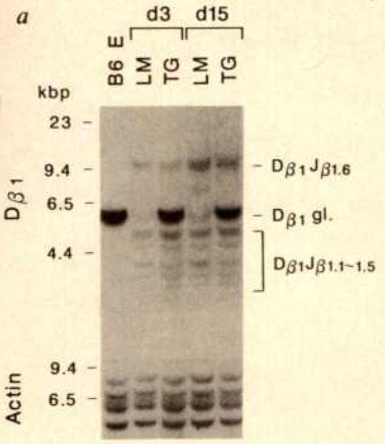

FIG. 3 Southern and northern blot analysis of DNA and RNA, respectively, from total thymocytes of DEC transgenic mice and nontransgenic littermates. *a*, Genomic DNA of thymocytes from 3- and 15-day-old DEC transgenic (TG) and nontransgenic (LM) mice and from C57BL/6 embryo (B/6 e) were digested with *Pvu*II, separated and transferred to nitrocellulose membrane. The Southern blot was hybridized with $D_{\beta 1}$ or actin probes. gl, Germ line DNA. DJ_β 1.1–1.6 bands were assigned according to ref. 29. *b*, RNA of thymocytes from 3- and 15-day-old DEC transgenic (TG) and nontransgenic (LM) mice was electrophoresed, transferred onto nitrocellulose membrane and hybridized to C_β or actin probes. The position of the 18S and 28S ribosomal RNA and that of $VDJC_\beta$ and DJC_β transcripts are indicated.

METHODS. Genomic DNA from thymocytes was purified as previously described[22], digested with *Pvu*II restriction endonuclease, separated (5 μg lane^{-1}) on a 0.6% agarose gel and transferred to GeneScreen Plus membrane (NEN) according to ref. 30. Hybridization with ^{32}P-labelled $D_{\beta 1}$ probe (*Pst*I fragment (1.06kb) from the clone encoding germ-line $D_{\beta 1}$ DNA[31]) and actin (*Pst*I fragment (0.8 kb) from a complementary DNA clone[32]) probes was carried out as previously described[30]. DNA probes were labelled by a random priming method[33]. The autoradiographic bands were detected and intensities quantitated using a FUJI Bioimage Analyzer BA100[34]. Total RNA was extracted using the guanidium thiocyanate–CsCl gradient method[35] and electrophoresed (10 μg lane^{-1}) through formaldehyde containing 1.2% agarose gel[22]. The RNA was transferred to GeneScreen Plus membrane and baked at 80 °C for 2 h. Northern blot hybridization with ^{32}P-labelled probes was performed under the same conditions as that of the Southern blot.

The above results indicate that introduction into the germ line of productively rearranged 'short', in contrast to 'long'[9], γ- and δ-chain transgenes severely hampers the development of αβ T cells. To further investigate the mechanism by which αβ T-cell differentiation is blocked, we analysed the DNA from thymocytes of young (3- and 15-day-old) DEC transgenic mice by the Southern blot method using a $D_{\beta 1}$ probe (Fig. 3*a*). In nontransgenic thymocytes, the germ-line $D_{\beta 1}$ band was barely detectable, reflecting the fact that incomplete *DJ* or complete *VDJ* rearrangements had taken place in most cells. By contrast, most $D_{\beta 1}$ gene segments remained in the germ-line configuration and the rest in the *DJ* state in the young transgenic mice (Fig. 3*a*). Consistent with the results of the Southern blot analysis, only low levels of full-size TCR β-chain messenger RNA (1.6 kb) were detected in transgenic thymocytes in comparison with nontransgenic thymocytes (Fig. 3*b*). Because *VJα* rearrangements were only partly inhibited in DEC transgenic mice (data not shown), these results strongly indicate that the primary mechanism by which the DEC γδ transgenes prevent αβ T-cell development is the blockage of complete *VDJβ* gene rearrangements. If this is indeed the case, why then do αβ T cells develop in older DEC transgenic mice? Northern blot analysis of αβ and γδ T-cell hybridomas from DEC transgenic mice shows that the levels of γ- and δ-chain transgene transcripts are lower in the former than in the latter (data not shown), suggesting that the level of γδ-TCR transcripts must be above a certain threshold to efficiently inhibit rearrangements of endogenous TCR genes. The αβ T cells observed in older DEC transgenic mice, then, are probably due to the accumulation of rare T cells in which the level of the transgene expression was below this threshold.

The drastic reduction in the number and the retardation of the appearance of αβ T cells in the DEC transgenic mice is perfectly consistent with the silencer model of T-cell development. The possibility must be considered, however, that αβ T cells are actually produced in these mice but are deleted in the thymus because of their coexpression of the transgene-encoded γδ TCR. Although nothing is known about the ligand specificity of the transgene ($V_5J_1C_1\gamma$ and $V_1D_2J_2C\delta$)-encoded TCR, it is conceivable that its expression in the αβ-cell lineage leads to negative selection of αβ T cells. To investigate this possibility, we crossed the DEC transgenic mice with αβ-transgenic mice[16]. If the silencer model is correct, then the introduction of functional α- and β-chain genes into DEC γδ-transgenic mice would be expected to restore αβ T cells. As shown in Fig. 4, ~80% of thymocytes from quadruple αβγδ-transgenic mice expressed αβ

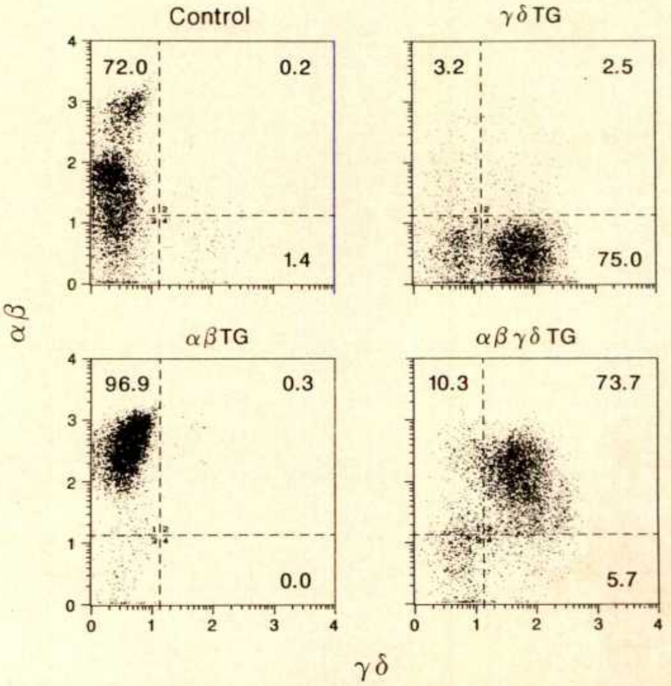

FIG. 4 Surface expression of γδ and αβ TCR by thymocytes from female γδ-, αβ- and αβγδ-transgenic mice. Thymocytes from 3-day-old DEC transgenic (γδ-TG), αβ-transgenic (αβ-TG), αβγδ-transgenic (αβγδ-TG) and nontransgenic (LM) mice were stained with FITC-coupled anti-γδ-chain monoclonal antibody 3A10 and biotinylated anti-αβ-chain monoclonal antibody H57-597 followed by phycoerythrin–streptavidin. The dot-plot histograms (3A10, horizontal axis; H57-597; vertical axis) obtained after cytofluorometric analysis are shown as well as the percentage of cells in each quadrant. Fluorescence intensity is expressed in $\log_{10}$ units.

METHODS. DEC transgenic females (H-2^b) were mated with αβ-transgenic male mice[16] (H-2^b) and their female progeny analysed 3 days after birth. Transgene expression was screened by staining thymocytes with anti-$V_{\beta 8}$ monoclonal antibody F23.1[36] and anti-αβ monoclonal antibody .57-597, or with anti-$V_{\gamma 5}$ monoclonal antibody F536 and anti-γδ monoclonal antibody 3A10. More than 95% of αβ T cells from αβ- and αβγδ-transgenic mice were $V^+_{\beta 8}$ and more than 98% of γδ T cells from γδ- and αβγδ-transgenic mice were $V^+_{\gamma 5}$.

TCR, whereas such cells were barely detectable in the thymus from DEC transgenic littermates. Furthermore, total numbers of $\alpha\beta$ thymocytes in $\alpha\beta$- and $\alpha\beta\gamma\delta$-transgenic mice were identical. Therefore we conclude that the drastic reduction in the number of $\alpha\beta$ T cells in the DEC transgenic mice is not due to intrathymic deletion of immature $\alpha\beta$ T cells.

Taken together, these observations strongly support the hypothesis that T-cell precursors are committed to generating $\alpha\beta$ T cells, independently of the rearrangement status of their γ-chain genes, and that this commitment involves activation of factor(s) that repress γ-chain gene transcription through interaction with a *cis*-regulatory element. Recently, Winoto and Baltimore[17] found that most extrachromosomal circular DNA generated by V_α-J_α joinings retain the δ-chain gene segments in the germ-line configuration and proposed that the control of δ-chain gene rearrangement plays a key part in the separation of $\alpha\beta$ and $\gamma\delta$ T-cell lineages. But, Takeshita *et al.*[18] reported that some 'α-circles' do contain rearranged δ-chain gene segments, suggesting that the control at the level of δ-chain gene rearrangement is insufficient.

It has previously been shown that, in contrast to C_1-associated γ-chain genes, $V_2J_2C_2\gamma$ genes are transcribed in $\alpha\beta$ T-cell clones and hybridomas[4,19-21]. The protein products of these genes have not been detected on the surface of freshly isolated T cells, however. Thus, the silencer model seems to apply to the separation of most, if not all, $\alpha\beta$ and $\gamma\delta$ T-cell lineages. □

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1. Allison, J. P., McIntyre, B. W. & Bloch, D. *J. Immun.* **129,** 2293–2300 (1982).
2. Meuer, S. C. *et al. Nature* **303,** 808–810 (1983).
3. Haskins, K. *et al. J. expl Med.* **157,** 1149–1169 (1983).
4. Saito, H. *et al. Nature* **309,** 757–762 (1984).
5. Hayday, A. C. *et al. Cell* **40,** 259–269 (1985).
6. Brenner, M. B. *et al. Nature* **322,** 145–149 (1986).
7. Pardoll, D. M. *et al. Nature* **326,** 79–81 (1987).
8. Allison, J. P. & Lanier, L. L. *Immun. Today* **8,** 293–296 (1987).
9. Ishida, I., Verbeek, S., Bonneville, M., Berns, A. & Tonegawa, S. *Cell* (manuscript submitted).
10. Alt, F. W., Blackwell, T. & Yancopoulos, G. D. *Science* **238,** 1079–1086 (1987).
11. Havran, W. L. & Allison, J. P. *Nature* **335,** 443–445 (1988).
12. Ito, K. *et al. Proc. natn. Acad. Sci. U.S.A.* **86,** 631–635 (1989).
13. Asarnow, D. M. *et al. Cell* **55,** 837–847 (1988).
14. Itohara, S., Nakanishi, N., Kanagawa, O., Kubo, R. & Tonegawa, S. *Proc. natn. Acad. Sci. U.S.A.* **86,** 5094–5098 (1989).
15. Kubo, R. T., Born, W., Kappler, J. W., Marrack, P. & Pigeon, M. *J. Immun.* **142,** 2736–2742 (1989).
16. Kisielow, P., Bluthmann, H., Staerz, U. D., Steinmetz, M. & von Boehmer, H. *Nature* **333,** 742–748 (1988).
17. Winoto, A. & Baltimore, D. *Nature* **338,** 430–432 (1989).
18. Takeshita, S., Toda, M. & Yamagishi, H. *EMBO J.* **8,** 3261–3270 (1989).
19. Heilig, J. S. & Tonegawa, S. *Nature* **322,** 836–840 (1986).
20. Reilly, E. B., Kranz, D. M., Tonegawa, S. & Eisen, H. N. *Nature* **321,** 878–880 (1986).
21. Rupp, F., Frech, G., Hengartner, H., Zinkernagel, R. M. & Joho, R. *Nature* **321,** 876–878 (1986).
22. Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1982).
23. Dillela, A. G. & Wood, S. L. C. *Focus* **7,** 1–5 (1985).
24. Takagaki, Y., Nakanishi, N., Ishida, I., Kanagawa, O. & Tonegawa, S. *J. Immun.* **142,** 2112–2121 (1989).
25. Bonneville, M. *et al. Proc. natn. Acad. Sci. U.S.A.* **86,** 5928–5932 (1989).
26. Garman, R. D., Doherty, P. J. & Raulet, D. H. *Cell* **45,** 733–742 (1986).
27. Iwashima, M., Green, A., Davis, M. M. & Chien, Y.-H. *Proc. natn. Acad. Sci. U.S.A.* **85,** 8161–8165 (1988).
28. Hogan, B., Costantini, F. & Lacy, E. *Manipulating the Mouse Embryo* (Cold Spring Harbor Laboratory, New York, 1986).
29. Born, W., Yagüe, J., Palmer, E., Kappler, J. & Marrack, P. *Proc. natn. Acad. Sci. U.S.A.* **82,** 2925–2929 (1985).
30. Chomzynski, P. & Qasba, P. K. *Biophys. Res. Commun.* **122,** 340–344 (1984).
31. Siu, G. *et al. Nature* **311,** 344–349 (1984).
32. Spiegelman, B. M., Frank, M. & Green, H. *J. biol. Chem.* **258,** 10083–10089 (1983).
33. Feinberg, A. P. & Vogelstein, B. *Analyt. Biochem.* **132,** 6–11 (1983).
34. Amemiya, Y. & Miyahara, J. *Nature* **336,** 89–90 (1988).
35. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. *Biochemistry* **18,** 5294–5299 (1980).
36. Staerz, J. D., Rammansee, H., Benedetto, J. & Bevan, M. J. *J. Immun.* **134,** 3994–4000 (1985).

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A protein binding to the J_κ recombination sequence of immunoglobulin genes contains a sequence related to the integrase motif

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SITE-SPECIFIC recombination requires conserved DNA sequences specific to each system, and system-specific proteins that recognize specific DNA sequences. The site-specific recombinases seem to fall into at least two families, based on their protein structure and chemistry of strand breakage (reviewed in ref. 1). One of these is the resolvase–invertase family, members of which seem to form a serine–phosphate linkage with DNA. Members of the other family, called the integrase family, contain a conserved tyrosine residue that forms a covalent linkage with the 3′-phosphate of DNA at the site of recombination. Structural comparison of integrases shows that these proteins share a highly conserved 40-residue motif[2]. *V-(D)-J* recombination of the immunoglobulin gene requires conserved recombination signal sequences (RS) of a heptamer CACTGTG and a T-rich nonamer GGTTTTTGT, which are separated by a spacer sequence of either 12 or 23 bases (reviewed in refs 3 and 4). We have recently purified, almost to homogeneity, a protein that specifically binds to the immunoglobulin J_κ RS containing the 23-base-pair spacer sequence[5]. By synthesizing probes on the basis of partial amino-acid sequences of the purified protein, we have now isolated and characterized the complementary DNA of this protein. The amino-acid sequence deduced from the cDNA sequence reveals that the J_κ RS-binding protein has a sequence similar to the 40-residue motif of integrases of phages, bacteria and yeast, indicating that this protein could be involved in *V-(D)-J* recombination as a recombinase.

The J_κ RS-binding protein was purified almost to homogeneity from the murine pre-B-cell line 38B9 (Fig. 1*a*) by the procedure of Hamaguchi *et al.*[5]. The J_κ RS-binding protein with a relative molecular mass (M_r) of 60,000 (60K) was expressed in lymphoid cell lines but not in nonlymphoid cell lines. The protein binds to the J_κ RS with a dissociation constant (K_d) of 1 nM. DNase-I footprinting analysis and the binding kinetic assay with several mutant probes strongly indicate that the protein directly interacts with the heptamer sequence and that a mutation in the heptamer greatly reduces the affinity of the J_κ RS-binding protein for DNA. Our initial attempt to determine the N-terminal amino-acid sequence was unsuccessful, probably because of modification of the N-terminal residue. The purified preparation was then digested with trypsin, and the resulting peptides were fractionated by reverse-phase HPLC (Fig. 1*b*). Twenty-three peptides were re-chromatographed and their amino-acid sequences were determined. Two oligodeoxyribonucleotide probes were synthesized on the basis of partial amino-acid sequences of the tryptic peptide 21 and used to screen a cDNA library derived from 38B9 poly(A)$^+$RNA (Fig. 2*a*, *b*). The probes hybridized with 2 of 1×10^6 colonies of the cDNA library. The two cDNA clones were purified and isolated by repeated screening. The clones designated as RBP-1 and RBP-2 contained 3.3- and 4-kilobase (kb) inserts, respectively. Restriction-site map-

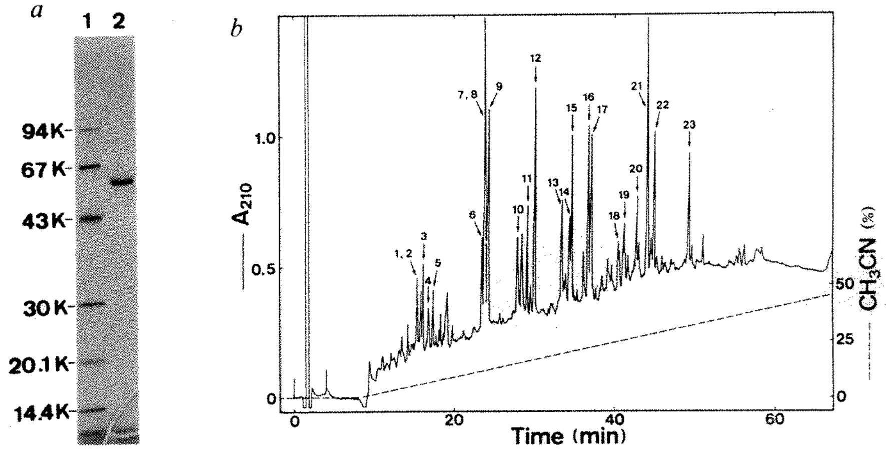

FIG. 1 Purification and partial amino-acid sequence analysis of J_κ RS-binding protein. *a*, Coomassie brilliant-blue stain of an SDS-polyacrylamide gel. Lane 1, mixtures of size markers; lane 2, purified J_κ RS-binding protein (0.5 μg). *b*, Reverse-phase HPLC of peptides formed by tryptic digestion of the purified J_κ RS-binding protein. Solid line and left-hand axis, absorbance at 210 nm (A210); broken line and right-hand axis, acetonitrile concentration. Peptides corresponding to peaks 1–23 were separated by re-chromatography and sequenced. All the amino-acid sequences thus determined agreed completely with regions of the amino-acid sequence deduced from the cDNA sequence (see Fig. 2*c*).

METHODS. A murine pre-B cell line 38B9 (ref. 13) which rearranges immunoglobulin genes during *in vitro* culture was maintained in RPMI 1640 medium with 10% (v/v) heat-inactivated FCS. The detailed protocol for the purification of the J_κ RS-binding protein is described in ref. 5. In brief, the crude nuclear extract from 38B9 (8×10^{10} cells) was fractionated by heparin-agarose column chromatography, followed by J_κ RS probe-coupled agarose column chromatography. Aliquots of fractions collected were assayed for binding activity to the J_κ RS probe in a gel-retardation assay. Each column chromatography was repeated twice. The method of SDS-PAGE was as in ref. 5. The dialysed preparation (200 pmol) of affinity-purified J_κ RS-binding protein were digested with trypsin. The digested peptides were fractionated by reverse-phase HPLC (Chemcosorb 30DS-H) with a linear gradient of acetonitrile in 0.1% trifluoroacetic acid. Each fraction was further separated by re-chromatography (μ-Bondaspher C-18 · 300A) to give a single absorbance peak. The amino-acid sequence of each peptide was determined using a gas-phase protein sequencer (Applied Biosystems).

a

```
Val-Trp-Phe-Gly-Asp-Val-Glu-Ala-Glu-Thr-Met-Tyr-Arg
   a-------------------------
                     b----------------------
```

b

```
a  3' ACC AAA CCA CTA CAA CTT CG 5'
            G   G   G   G   C
                T       T
                C       C
```

```
b  3' CTT CGA CTT TGA TAC AT 5'
        C   G   C   G
            T       T
            C       C
```

c

```
                                                                                                                                         5'......TAGTA    -1
ATGCCCTCCGGTTTTCCTCAGTCTCCACGTACGTCCCCGAGGGCGCGTCCCAAAACCCGGATAACCGGAGCGCTCCCCATGGACTACTCGGAGGGCTTGTCCGCGGAGGAGCGGCCTGCGCATGCTCCATCGGCGGGGAAGTTTGGTGAG   150
 M  P  S  G  F  P  Q  S  P  R  T  S  P  R  A  R  P  K  T  R  I  T  G  A  L  P  M  D  Y  S  E  G  L  S  A  E  E  R  P  A  H  A  P  S  A  G  K  F  G  E     50
                                                                                                                                             8--------
CGGCCTCCACCCAAACGACTCACTAGGGAAGCTATGCGAAATTATTTAAAAGAACGAGGGGATCAAACAGTGCTCATTCTTCATGCAAAAGTTGCACAGAAGTCTTACGGAAATGAAAAACGATTTTTTTGCCCTCCTCCTTGTGTGTAT   300
 R  P  P  P  K  R  L  T  R  E  A  M  R  N  Y  L  K  E  R  G  D  Q  T  V  L  I  L  H  A  K  V  A  Q  K  S  Y  G  N  E  K  R  F  F  C  P  P  P  C  V  Y    100
---------------            2-----------6-----------14-------------------------------------            5--------------------
CTTATGGGCAGTGGTTGGAAGAAAAAAAAAGAGCAAATGGAACGAGATGGATGTTCTGAACAAGAGTCTCAACCCTGTGCGTTTATTGGAATAGGAAACAGTGACCAAGAAATGCAGCAGCTGAACTTGGAAGGGAAGAACTACTGCACA   450
 L  M  G  S  G  W  K  K  K  K  E  Q  M  E  R  D  G  C  S  E  Q  E  S  Q  P  C  A  F  I  G  I  G  N  S  D  Q  E  M  Q  Q  L  N  L  E  G  K  N  Y  C  T    150
                              4--------------19-------------------------------------------------
GCCAAAACATTGTACATATCTGATTCAGACAAGAGAAAACATTTCATGTTGTCTGTAAAGATGTTCTATGGCAACAGCGATGACATTGGTGTGTTCCTCAGCAAGCGGATAAAGGTCATCTCCAAACCCTCCAAAAAGAAGCAGTCACTG   600
 A  K  T  L  Y  I  S  D  S  D  K  R  K  H  F  M  L  S  V  K  M  F  Y  G  N  S  D  D  I  G  V  F  L  S  K  R  I  K  V  I  S  K  P  S  K  K  K  Q  S  L    200
      11--------------•-------------15-------------         22----------------------------------------                                       1--------
AAGAATGCTGACTTGTGCATTGCTTCAGGAACGAAGGTGGCACTGTTCAATCGCCTTCGGTCCCAGACAGTTAGTACCAGGTACCTGCATGTAGAAGGAGGGAATTTCCACGCCAGTTCACAACAGTGGGGAGCATTTTACATCCATCTC   750
 K  N  A  D  L  C  I  A  S  G  T  K  V  A  L  F  N  R  L  R  S  Q  T  V  S  T  R  Y  L  H  V  E  G  G  N  F  H  A  S  S  Q  Q  W  G  A  F  Y  I  H  L    250
---                                 13----------------      3--------------------       <===
TTGGACGACGACGAGTCGGAAGGAGAGGAGTTCACAGTTAGAGATGGCTACATCCATTACGGGCAGACTGTCAAGCTTGTGTGCTCAGTGACTGGCATGGCACTCCCAAGATTGATAATTAGGAAAGTTGATAAGCAGACGGCATTACTG   900
 L  D  D  D  E  S  E  G  E  E  F  T  V  R  D  G  Y  I  H  Y  G  Q  T  V  K  L  V  C  S  V  T  G  M  A  L  P  R  L  I  I  R  K  V  D  K  Q  T  A  L  L    300
                                          12--------===>-------------------                                                            16-------------
GATGCAGACGACCCTGTATCACAACTCCACAAATGTGCATTTTACCTTAAGGATACAGAAAGAATGTACTTGTGCCTTTCTCAAGAAAGAATAATCCAATTTCAGGCCACTCCATGTCCAAAAGAACAAAATAAGGAAATGATAAACGAT  1050
 D  A  D  D  P  V  S  Q  L  H  K  C  A  F  Y  L  K  D  T  E  R  M  Y  L  C  L  S  Q  E  R  I  I  Q  F  Q  A  T  P  C  P  K  E  Q  N  K  E  M  I  N  D    350
---------------------------------                              20-------------------------                                             18-------------
GGAGCTTCCTGGACAATCATTAGCACAGACAAGGCAGAATACACGTTCTATGAGGGAATGGGCCCCGTCCTTGCCCCAGTCACCCCTGTGCCTGTCGTAGAAAGTCTTCAGTTGAATGGCGGCGGGGACGTAGCAATGCTTGAACTTACA  1200
 G  A  S  W  T  I  I  S  T  D  K  A  E  Y  T  F  Y  E  G  M  G  P  V  L  A  P  V  T  P  V  P  V  V  E  S  L  Q  L  N  G  G  G  D  V  A  M  L  E  L  T    400
---------------------------------
GGACAAAACTTTACTCCAAATTTACGAGTGTGGTTTGGGGATGTAGAAGCCGAAACAATGTACAGATGTGGAGAGAGCATGCTCTGTGTGGTCCCAGACATTTCTGCATTCCGGGAAGGTTGGAGATGGGTCCGCCAGCCAGTCCAGGTT  1350
 G  Q  N  F  T  P  N  L  R  V  W  F  G  D  V  E  A  E  T  M  Y  R  C  G  E  S  M  L  C  V  V  P  D  I  S  A  F  R  E  G  W  R  W  V  R  Q  P  V  Q  V    450
                           21=====================================23-------------------------------------------   9-----------7--------17-------------
CCAGTGACTTTGGTCCGAAATGACGGGGTCATTTACTCCACCAGCCTTACCTTCACCTACACACCAGAGCCAGGGCCGAGGCCACACTGCAGCGCTGCAGGAGCGATTCTCAGAGCCAACTCCAGCCAAGTGCCCTCCAATGAGTCAAAC  1500
 P  V  T  L  V  R  N  D  G  V  I  Y  S  T  S  L  T  F  T  Y  T  P  E  P  G  P  R  P  H  C  S  A  A  G  A  I  L  R  A  N  S  S  Q  V  P  S  N  E  S  N    500
------------------                                                                                                10----------------------------------
ACAAACAGCGAGGGGAATTACACAAATGCCAGCACAAATTCTACCAGTGTCACATCGTCCACAGCAACCGTGGTGTCCTAACTACCGTCTTTTTGCTGGGACTGAAACTGACTTGAATGCAGCAAAAAGTTGACAGAGAAAGGCAAGGAT  1650
 T  N  S  E  G  N  Y  T  N  A  S  T  N  S  T  S  V  T  S  S  T  A  T  V  V  S  *                                                                        526
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AAAGTGAACAATCTTTTGTGGTTTCTTGGGGAAACGTTTCGTACCAGGTGATCTATTCAAAACTGCCAGTGCAGGTGTGACATGCAGAGGCCACAGTACAATGAACAACATCAGAATGCACAGGCTGATGGAAATCCAGGTTTTCAGCTG  1800
TCCCTCTCACACACACCCCTTTTTGGTTGTTTTGGTTTGGTTTTGATTTAATGGCCAGGAAACAGATATGTGGCTGGAGCACAGGCTAAGCTAGAAGACACAAACCTAACATAGACTTATGGACCAAGGGACTCGCATAAGCTTTAGTGA  1950
AAGGTACATTTTCACCATGCCTTTTTTATATCACAGTGTGTACTACACCTTGTTACCAAACAGGTTGCGCTCCCCACCCTTTGAGGGTTGGCTCTAAAGTACATTCAGGTTCGAGCCTCACCATGTTTGTTCAGTCTGAACACTGAACAC  2100
TTCCAGGTTCTTGTGGTCTAAGGCTGGAACTTTTTTAAGCTGAAGTCTTATGACTTTTTCATAAGTTGGAATTATTTTGATTTCAGCAAGTCAAATTTTGTAAAGGCCTGCATATTTTTTTTAAGATTATATGAAGTCTGCAAAAGCTTT  2250
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CATTTAGCCCTTTCTGAGTTCATGGCAGTGAGCACCGCCGTTCGAAGGCTATGCGCCTCTGCCTGTTCATCAGTTCATCTGTGTTCCAGGATTTGTTCAGGTTTCCCCTTCCCCCAAATCTTGTACATAACTTGTATTATGTGTAAGTTA  2550
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TTTATTGTATGTTTTCCCTTTTTTAGCCTAGCCAGAACATCATTATAATTTTAAAGACCATAAGATGGCTTTTATTAGATGATAACTAAATAAACTGAACTAAGTAGCCAGAAGACAGTACCTTAGAGACTGATAGTTGTAAGTTTATA  2850
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CCAGATTCATTTTGTTTCCATCCCTGCACTTTGGTTATTCCTCGTTCAGTTTCTTGGGAATTGAAGCAGACTCATTGGCTACATTTAGTACAAGAACCACATGTGTGATGTTAAAGGACACAGTCTAGTGATGCCATCATCCTTGAGTA  3150
AAAACTACCTCTAGATTGTGGTAAGCTTTTACTGTCCCTTAAAACAGGAGCCACTAGTACCTTACGAATGCAGACTGTAACTTCTATGGCATTTTCTGGTGTCTTTCTGGCTCTCTGGGCTTCTGAAAGTTTTCACGTAGACCCTTAGAC  3300
TCCTCTGTCAGGACCATTGCTTTCCAGCGTTGCTGCTTTCACTCGCTGTCTCGCAGACTGTACCACTGTAAGTGCCACTGCGAACATCTTTAAACGGCCTCCTCCTTGGCTTGTCAAGGGGAAGGCCACCTGTAAGATGTGGTGGTGACA  3450
GCCTTCCTGTCCACCCCAGGCTGCTCCTTACCAGCTGTTCTAGCCGATTCTCTTCAGATCAGCTACAGCCCACCTCCTCCCCGCCCCGCCTCACCCCTCCAGGCTGTCTCAGTGGTCCAGGGCAGAGCTCACTGTGGCCTTACTGATCTA  3600
TCTCTGCAGTAACTGTTCTCCCTCAGTTGCGATGGAAGCAGGCCCCTGCGATCTGTTCAGAGCCTGAGCAGTTTGCAGTTCCGTTGTGCTTTCTGAGGTCCTAGATTCAGGATGTAGTTAAGTCGATAACTGAGAACAGATGAGATGCTT  3750
TTAAAGAACAAACTCTTCCTTACCCAGCTTTGTAGCTTAAAGTAAGTTTTCAAGTTCATGGCTTTATTAAAATGAACTTTGACTTTTTAAAACTGCTTATATCTCATTTCGGTTCTTTTGTTTATTTTAGTAAGAATTGAAACAGACTAT  3900
AAGTAGTTTTCCTGTTTGTTTGTTTGTTTGTTTTTTACAAAGTTCAAGAATGTAACAATAAAGGCATTTTGCTATGGTTCTATGTC......3'                                                       3986
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FIG. 2 Nucleotide sequence and corresponding amino-acid sequence of J_κ RS-binding protein. *a*, Amino-acid sequence of tryptic fragment 21. Sequences used for construction of probes a and b are indicated. *b*, Synthetic oligodeoxyribonucleotide mixtures used as probes a and b. *c*, Nucleotide sequence and predicted amino-acid sequence (single-letter code) of J_κ RS-binding protein. Thick and thin underlines indicate peptide 21 and other peptides sequenced from the purified protein, respectively. Poly(A)-addition signal sequences are boxed. Asterisk indicates termination codon. The boundaries of the integrase motif region (Fig. 4) are indicated by two horizontal arrows.

METHODS. A cDNA library linked to the SV40 early promoter was constructed from poly(A)$^+$RNA of 38B9 cells, as described, by Okayama and Berg[14]. *E. coli* AG-1 transformants were screened with ^{32}P-labelled oligodeoxynucleotide probes a and b by the method of Hanahan and Meselson[15]. Nucleotide sequences were determined by the dideoxy method using plasmid pUC vector[16]. Oligonucleotides were synthesized by an automated synthesizer (Applied Biosystems).

ping and nucleotide sequence determination showed that the two clones are identical, except that RBP-2 has an extra 550-base-pair (bp) sequence at the 5′ end and uses the 140-bp downstream poly(A) addition site.

Nucleotide-sequence determination of the cDNA revealed an open reading frame that encodes 526 amino-acid residues (Fig. 2*c*). All the 23 partial amino-acid sequences determined (Fig. 1*b*) were identified in this amino-acid sequence deduced from the cDNA sequence. The calculated M_r of the J_κ RS-binding protein as 57K (including the initiating methionine), in reasonable agreement with the value of 60K estimated by SDS-PAGE. Of the total 4,000-bp insert, the 3′-noncoding region occupies 2,400 nucleotides excluding the poly(dA) tract; two polyadenylation signal sequences are located 30 and 170 nucleotides upstream of the poly(dA) tract of RBP-2. The size of the J_κ RS-binding protein messenger RNA (4.5 kb) of 38B9 cells,

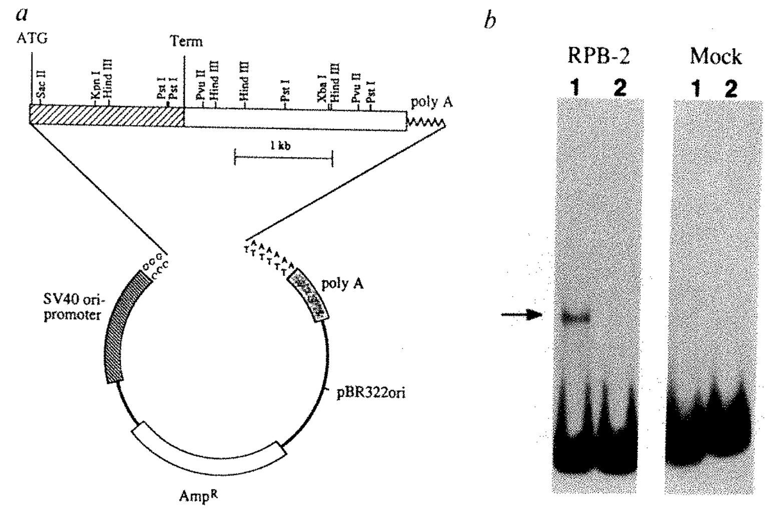
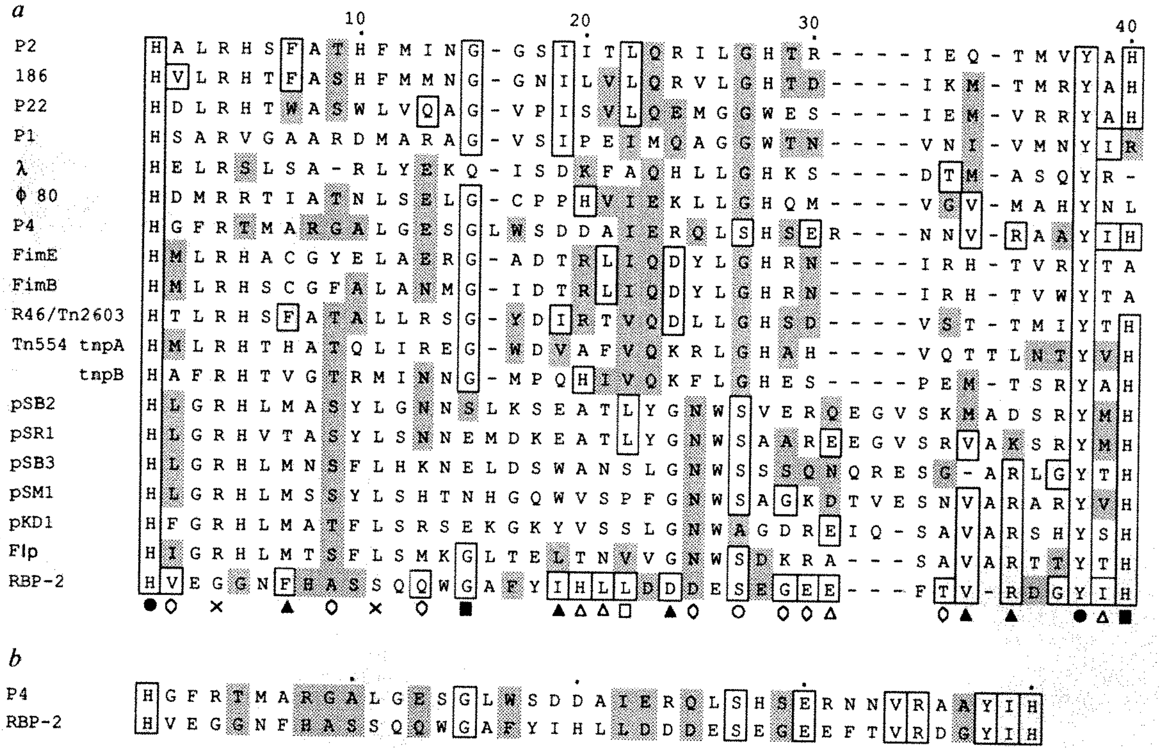


FIG. 3 *a*, Restriction map and structure of RBP-2 cDNA clone. The original RBP-2 clone in Okayama-Berg expression vector was used. Solid and zig-zag lines indicate vector and poly(A) tails, respectively. Black and open rectangles indicate coding and untranslated regions, respectively. *b*, Expression of RBP-2 cDNA in COS-7 cells. Nuclear extracts from RBP-2 and mock-transfected COS-7 cells were purified in a DNA-affinity column[5], and were assayed for binding to the J_κ RS probe (lane 1) or its single-base substitution mutant probe, J_κ 1P3 (lane 2) in a gel-retardation assay[5]. Term, termination site.

METHODS. COS-7 (1.5×10^7 cells) maintained in DMEM medium with 5% FCS were transfected and cultured as previously described[17]. After 48 h of culture, RBP-2 and mock-transfected COS-7 cells were collected. Their nuclear extracts were prepared and dialysed against buffer A (50 mM NaCl, 20 mM Tris-HCl buffer, pH 7.4, 1 mM EDTA, 1 mM DTT, 30% glycerol) as previously described[5]. Each nuclear extract was fractionated by affinity chromatography on a column containing 1 ml packed J_κ RS DNA-coupled agarose[5]. After sample application, the column was washed with five volumes of buffer A, and then eluted with buffer A containing 250 mM NaCl. For the gel-retardation assay, aliquots of eluted fractions containing the same amount of protein were incubated in reaction mixture (30 µl total), containing 50 mM NaCl, 5 mM Tris-HCl (pH 7.5), 1 mM EDTA, 1 mM DTT, 30% glycerol with 2 ng radiolabelled probe, 1 µg poly(dI), poly(dC) for 30 min at 20 °C. The incubated sample was analysed by 4% PAGE as previously described[5].



a

10 20 30 40

P2 H A L R H S F A T H F M I N G - G S I I T L Q R I L G H T R - - - - I E Q - T M V Y A H

186 H V L R H T F A S H F M M N G - G N I L V L Q R V L G H T D - - - - I K M - T M R Y A H

P22 H D L R H T W A S W L V Q A G - V P I S V L Q E M G G W E S - - - - I E M - V R R Y A H

P1 H S A R V G A A R D M A R A G - V S I P E I M Q A G G W T N - - - - V N I - V M N Y I R

λ H E L R S L S A - R L Y E K Q - I S D K F A Q H L L G H K S - - - - D T M - A S Q Y R -

Φ 80 H D M R R T I A T N L S E L G - C P P H V I E K L L G H Q M - - - - V G V - M A H Y N L

P4 H G F R T M A R G A L G E S G L W S D D A I E R Q L S H S E R - - - N N V - R A A Y I H

FimE H M L R H A C G Y E L A E R G - A D T R L I Q D Y L G H R N - - - - I R H - T V R Y T A

FimB H M L R H S C G F A L A N M G - I D T R L I Q D Y L G H R N - - - - I R H - T V W Y T A

R46/Tn2603 H T L R H S F A T A L L R S G - Y D I R T V Q D L L G H S D - - - - V S T - T M I Y T H

Tn554 tnpA H M L R H T H A T Q L I R E G - W D V A F V Q K R L G H A H - - - - V Q T T L N T Y V H

tnpB H A F R H T V G T R M I N N G - M P Q H I V Q K F L G H E S - - - - P E M - T S R Y A H

pSB2 H L G R H L M A S Y L G N N S L K S E A T L Y G N W S V E R Q E G V S K M A D S R Y M H

pSR1 H L G R H V T A S Y L S N N E M D K E A T L Y G N W S A A R E E G V S R V A K S R Y M H

pSB3 H L G R H L M N S F L H K N E L D S W A N S L G N W S S S Q N Q R E S G - A R L G Y T H

pSM1 H L G R H L M S S Y L S H T N H G Q W V S P F G N W S A G K D T V E S N V A R A R Y V H

pKD1 H F G R H L M A T F L S R S E K G K Y V S S L G N W A G D R E I Q - S A V A R S H Y S H

Flp H I G R H L M T S F L S M K G L T E L T N V V G N W S D K R A - - - S A V A R T T Y T H

RBP-2 H V E G G N F H A S S Q Q W G A F Y I H L L D D D E S E G E E - - - F T V - R D G Y I H

● ⬡ × ▲ ⬡ × ⬡ ■ ▲ △ △ □ ▲ ⬡ ○ ⬡ ⬡ △ ⬡ ▲ ▲ ● △ ■

b

P4 H G F R T M A R G A L G E S G L W S D D A I E R Q L S H S E R N N V R A A Y I H

RBP-2 H V E G G N F H A S S Q Q W G A F Y I H L L D D D E S E G E E F T V R D G Y I H

FIG. 4 Comparison of the 40-residue motif sequences of the J_κ RS-binding protein and recombinases of the integrase family (single-letter amino-acid code). *a*, Alignment with all the known sequences. *b*, Alignment with P4 integrase. Only residues that are conserved between the J_κ RS-binding protein (RBP-2) and any of the other proteins are boxed. Residues that belong to the same exchange groups with those of RBP-2 are shaded. Extents of conservations are indicated by closed and open symbols at the bottom, excluding and including, respectively, residues of the same exchange group. Circles, 100%, rectangles, >68%; triangles, >21%. Residues with less than two identical members but more than seven members of identical exchange groups are indicated by open hexagons. Residues that are conserved >60% among the other recombinases, but not conserved in RBP-2, are shown by crosses. Origins of integrase sequences are; Phages P2 (ref. 2), 186 (ref. 2), P22 (ref. 2), P1 (ref. 2), λ (ref. 2), φ80 (ref. 2), and P4 (ref. 18); FimE and FimB proteins (ref. 19); transposons Tn*2603* (ref. 20) and Tn*554* (ref. 21); plasmids pSB2 (ref. 22), pSR1 (ref. 23), pSB3 (ref. 24), pSM1 (ref. 22), and pKD1 (ref. 25); and Flp protein (ref. 26). Different reading frames for Tn*554* indicated by tnpA and tnpB.

estimated by northern-blot hybridization analysis, is in general agreement with the size of the RBP-2 encoded protein if the additional poly(A) tract is considered (data not shown).

To confirm that the cDNA clones isolated encode the J_κ RS-binding protein, COS-7 cells were transfected with RBP-2 cDNA in an expression plasmid linked to the simian virus 40 (SV40) early promoter (Fig. 3*a*). After 48 h of culture, the nuclear extract of transfected COS-7 cells was prepared and fractionated by affinity chromatography using agarose coupled to J_κ RS DNA[5]. Aliquots of fractions bound to the affinity column were assayed for binding to the J_κ RS probe in a gel-regardation assay. Binding was detected in the extract of RBP-2-transfected COS-7 cells but not in mock-transfected COS-7 cells (Fig. 3*b*). Furthermore, a marked reduction in the binding affinity was observed when a mutant probe (J_κ 1P3 probe) with a single base substitution of the third G in the heptamer was used (Fig. 3*b*), consistent with our previous observation that the GTG sequence of the heptamer interacts with the J_κ RS-binding protein[5]. These results strongly indicate that the RBP-2 cDNA sequence encodes the J_κ RS-binding protein. This protein has a basic residue cluster (Lys-Lys-Lys-Lys for residues 107–110) which often serves as a nuclear-localization sequence[6].

A computer survey did not reveal any other proteins that have extensive sequence homology with the overall sequence of the J_κ RS-binding protein. The protein does not seem to have any DNA-binding domains such as zinc-finger[7], helix-turn-helix[8], or leucine-zipper[9] structures. But the J_κ RS-binding protein contains a sequence similar to the 40-residue motif of the integrase family that includes λ phase integrase, fimbriae switch recombinase of *Escherichia coli* and yeast Flp recombinase[2] (Fig. 4*a*). In the 40-residue motif, three residues (His 1, Arg 4 and Tyr 38) are perfectly conserved among all the 18 integrase-family proteins so far sequenced. The conserved tyrosine residue (Tyr 38), of Flp[10,11] and λ phage integrase[12] is involved in the catalytic reaction of the integrase. The J_κ RS-binding protein conserves the His 1 and Tyr 38 residues, but not the Arg 4 residue. In addition, Leu 11 is >60% conserved among the other recombinases, but is not conserved in the J_κ RS-binding protein. Nonetheless, the J_κ RS-binding protein contains two highly (>68%) conserved residues (Gly 15 and His 40) and seven moderately (>21%) conserved residues within the 40-residue motif. If we regard residues in the same exchange group as conserved, 21 out of 40 residues of the motif region of the J_κ RS-binding protein are >32% conserved among all the 19 proteins.

Out of the other 18 recombinases, the J_κ RS-binding protein has the highest homology with the P4 integrase (Fig. 4*b*). The 40-residue motifs of these two proteins can be aligned without any deletions and insertions, and share 9 identical and 11 same-family residues. Furthermore, the first 16 residues of the recognition sequence (GAGTCCGGCCTTCGGCACCA) for the P4 integrase[18] has some homology with the sequence of the heptamer-nonamer of the immunoglobulin gene, that is, GAGTC(A)CGGTTTTTGT.

In view of the large evolutionary distance between bacteriophages and mouse, such homology indicates that this motif in the J_κ RS-binding protein could be conserved to maintain the function of the protein as a recombinase. It is of interest that the primary structures of recombinases of the integrase family are divergent except for this homologous 40-residue motif, which seems to be a catalytic domain but not a binding domain for DNA. *In vitro* studies of site-specific recombination indicate that the reaction is essentially catalysed by a single protein that seems to be required in stoichiometric rather than catalytic amounts, although additional accessory proteins are required in some cases[1]. Experiments are in progress to examine whether the J_κ RS-binding protein catalyses or stimulates *V*-(*D*)-*J* recombination, although other accessory binding proteins, such as integration host factor[1], could be required simultaneously for efficient recombination. □

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1. Craig, N. L. *A. Rev. Genet.* **22,** 77–105 (1988).
2. Argos, P. *et al. EMBO J.* **5,** 433–440 (1986).
3. Tonegawa, S. *Nature* **302,** 575–581 (1983).
4. Honjo, T. *A. Rev. Immun.* **1,** 499–518 (1983).
5. Hamaguchi, Y. *et al. Nucleic Acids Res.* (in the press).
6. Van Etten, R. A., Jackson, P. & Baltimore, D. *Cell* **58,** 669–678 (1989).
7. Miller, J., McLachlan, A. D. & Klug, A. *EMBO J.* **4,** 1609–1614 (1985).
8. Pabo C. O. & Sauer, R. T. *A. Rev. Biochem.* **53,** 293–321 (1984).
9. Landschulz, W. H., Johnson, P. F. & McKnight, S. L. *Science* **240,** 1759–1764 (1988).
10. Parsons, R. L., Prasad, P. V., Harshey, R. M. & Jayaram, M. *Molec. cell. Biol.* **8,** 3303–3310 (1988).
11. Pasad, P. V., Young, L.-J. & Jayaram, M. *Proc. natn. Acad. Sci. U.S.A.* **84,** 2189–2193 (1987).
12. Pargellis, C. A., Nunes-Duby, S. E., Moitoso de Vargas, L. & Landy, A. *J. biol. Chem.* **263,** 7678–7685 (1988).
13. Alt, F., Rosenberg, N., Lewis, S., Thomas, E. & Baltimore, D. *Cell* **27,** 381–390 (1981).
14. Okayama, H. & Berg, P. *Molec. cell. Biol.* **2,** 161–170 (1982).
15. Hanahan, D. & Meselson, M. *Gene* **10,** 63–67 (1980).
16. Hattori, M. & Sakai, Y. *Analyt. Biochem.* **152,** 232–239 (1986).
17. Nikaido, T. *et al. Nature* **311,** 631–635 (1984).
18. Pierson III, L. S. & Kahn, M. L. *J. molec. Biol.* **196,** 487–496 (1987).
19. Klemm, P. *EMBO J.* **5,** 1389–1393 (1986).
20. Hall, R. M. & Vockler, C. *Nucleic Acids Res.* **15,** 7491–7501 (1987).
21. Murphy, E., Huwyler, L. & de Freire Bastos, M. d-C. *EMBO J.* **4,** 3357–3365 (1985).
22. Utatsu, I., Sakamoto, S., Imura, T. & Toh-E, A. *J. Bact.* **169,** 5537–5545 (1987).
23. Araki, H. *et al. J. molec. Biol.* **182,** 191–203 (1985).
24. Toh-e, A. & Utatsu, I. *Nucleic Acids Res.* **13,** 4267–4283 (1985).
25. Chen, X. J., Saliola, M., Falcone, C., Bianchi, M. M. & Fukuhara, H. *Nucleic Acids Res.* **14,** 4471–4481 (1986).
26. Hartley, J. L. & Donelson, J. E. *Nature* **286,** 860–864 (1980).

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Genetic evidence that *ZFY* is not the testis-determining factor

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IN mammals, the testis determining gene (*TDF*), present on the Y chromosome, induces the undifferentiated gonads to form testes[1]. The position of *TDF* on the human Y chromosome has been defined by analysing the genomes of XX males and XY females[2], generated by abnormal genetic exchange between the X and Y chromosomes in male meiosis[3]. In this way *TDF* has been localized close to the pseudoautosomal region shared by the sex chromosomes, in the distal Y-specific region. A recently cloned human gene, *ZFY*, has many features indicating that it is *TDF*[4–6]. For example, *ZFY* encodes a protein with many features of a transcription factor including a domain with multiple 'zinc-finger' motifs[4–6]. Less consistent with *ZFY* being *TDF*, however, is the presence of a very similar gene, *ZFX*, on the X chromosome[4,7], and the presence of a sequence related to *ZFY* on autosomes in marsupials[8]. We now report on analysis of XX males lacking *ZFY*. In these individuals, the male phenotype could be explained by a mutation in a gene 'downstream' of *ZFY* in the sex-determining hierarchy; but in that case there should be no exchange of material between the X and Y chromosomes. We find on the contrary that in 4 XX males lacking *ZFY*, there is exchange of Y-specific sequences next to the pseudoautosomal boundary, redefining the region in which *TDF* must lie.

By studying the sequences present in an XX male (LGL203) and absent in an XY female (WHT1013), Page *et al.* had previously localized *TDF* to within 140 kilobases (kb) (ref. 4 and Fig. 1). The latter patient, however, was not a typical exchange XY female, but had a translocation between the Y chromosome and chromosome 22. The sequences between the breakpoints in these patients were isolated and the gene *ZFY* was identified in this region.

TABLE 1 Mapping of breakpoints in XX sex-reversed individuals

Patient	Origin	Karyotype	Hypospadia	Internal genitalia	Testis biopsy	HFO.2 (Y-boundary)	H2.1	P0.9	Probe E1.3	27A	GMGY3	pMF-1 (*ZFY*)
ZM	Algeria	46 XX	Anterior	Vaginal pouch	Leydig cells Sertoli cells No spermatogonia	+	+	+	–	–	–	–
MB	France	46 XX	None	Normal male†	Leydig cells Sertoli cells No spermatogonia	+	+	+	–	–	–	–
DL*	France	46 XX	Posterior	Uterus	Bilateral ovotestis	+	+	+	–	–	–	–
TL*	France	46 XX	Anterior	Normal male	Sertoli cells No spermatogonia	+	+	+	–	–	–	–

* Familial case.

† Patient presented with bilateral cryptorchidism.

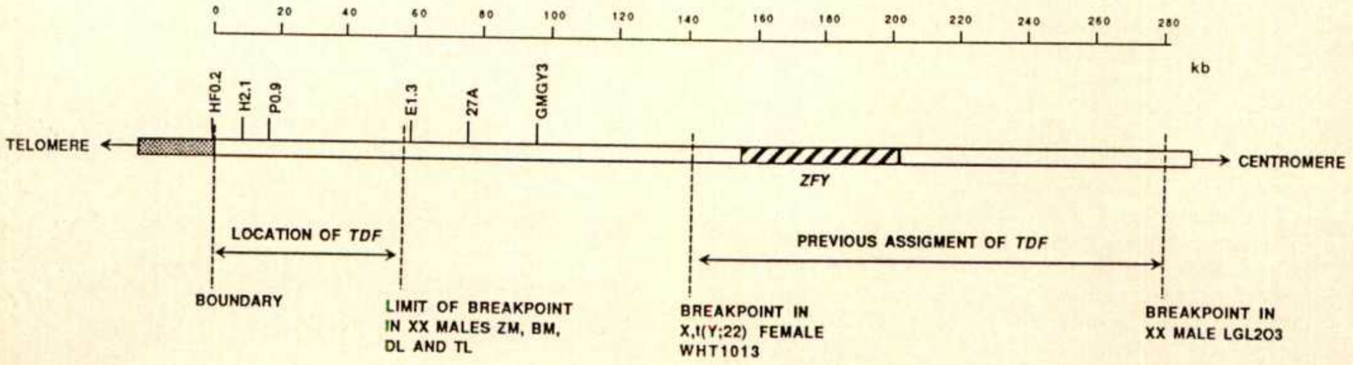
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
kb
HF0.2
H2.1
P0.9
E1.3
27A
GMGY3
TELOMERE
CENTROMERE
ZFY
LOCATION OF TDF
PREVIOUS ASSIGMENT OF TDF
BOUNDARY
LIMIT OF BREAKPOINT IN XX MALES ZM, BM, DL AND TL
BREAKPOINT IN X,t(Y;22) FEMALE WHT1013
BREAKPOINT IN XX MALE LGL203

FIG. 1 Map of distal short arm of Y chromosome: stippled region at left is the pseudoautosomal region. Cross-hatched region shows the position of *ZFY* identified by Page *et al.*[4]. The breakpoints in the XX male and XY female used in the localization of *ZFY* are shown. Individual probes used to define the breakpoints in the XX males reported here were isolated from overlapping clones obtained while walking along the Y chromosome[9], except probes 27a and GMGY3, which were isolated previously[15,16]. Hf0.2 derives from the pseudoautosomal region and is present on both X and Y chromosomes. The clones H2.1, P0.9, E1.3, 27a and GMGY3 are specific for the Y chromosome.

In previous reports we have described the isolation and structure of the boundary between the pseudoautosomal region and the sex chromosome-specific regions[9]. If *ZFY* is *TDF*, it should be predicted that all exchange XX males carry *ZFY*, whereas all XX males lacking *ZFY* are due to mutations elsewhere in the genome. The latter class cannot be derived by terminal X–Y exchange and should consequently lack the Y chromosome-derived pseudoautosomal boundary. Using a polymerase chain reaction (PCR)-based assay, 14 *ZFY*-negative XX males were screened for the presence of the Y chromosome-derived pseudoautosomal boundary: three XX males and one XX intersex individual were found to have the Y pseudoautosomal boundary (Fig. 2). Repeating these experiments by Southern blotting confirmed that all four sex-reversed individuals were positive for both the X and Y boundaries and were *ZFY*-negative (Fig. 3). The relative signal intensities between the X and Y boundary fragments excludes mosaicism and chimaerism as explanations of the results. The possibility that the boundary position is polymorphic is also unlikely as the boundary structure and position is invariant in more than 150 individuals tested; moreover, the Y boundary is in the same position in the great apes (ref. 9 and unpublished observations). The position of the exchanges in the four exceptional XX sex-reversed individuals was mapped by testing for reaction with a series of probes located between the boundary and *ZFY* (Fig. 3). The results summarized in Table 1 and in Fig. 1 show that all the breakpoints are clustered between the sequences defined by probes P0.9 and E1.3. The region of the Y chromosome present in these sex-reversed individuals does not include the region defined by Page *et al.*[4] as the sex-determining region. The simplest interpretation of these results is that *TDF* lies within 60 kb of the pseudoauto-

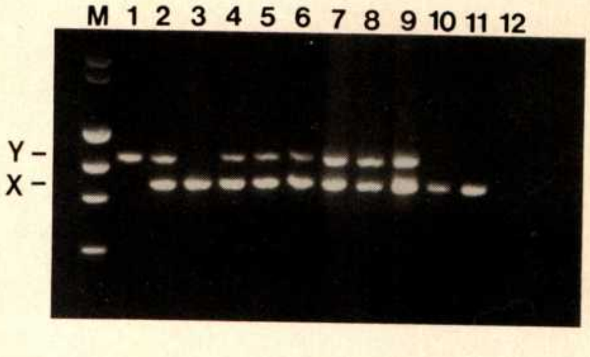

FIG. 2 PCR analysis of X and Y boundaries. Oligonucleotides specific for X- or Y-chromosomal sequences were used together with an oligonucleotide from the pseudoautosomal region as primers in PCR reactions[17]. The Y-specific fragment is 1.1 kb and the X-specific fragment, 0.95 kb. The size markers in lane M are 2.3- and 2.0-kb λ-*Hin*dIII fragments, and 1.35-, 1.0-, 0.87- and 0.60-kb ϕX173 *Hae*III fragments. PCR was performed on 1 μg genomic DNA from the following sources: (1) 3E7, a mouse–human hybrid containing the human Y chromosome as its sole contribution of human DNA[18]; (2) a normal XY male; (3) a normal XX female; (4) an XX male, SB, positive for *ZFY*; (5) an XX male J–JM positive for *ZFY*; (6) XX male, MB, negative for *ZFY*; (7) XX intersex, TL, negative for *ZFY*; (8) XX male, DL, brother of TL, negative for *ZFY*; (9) XX male, ZM, negative for *ZFY*; (10) XX male, PP, negative for *ZFY* and Y boundary; (11) XX male, LS, negative for *ZFY* and Y boundary; (12) no DNA added, control. Of fourteen *ZFY* XX males or intersex individuals analysed, four—MB, TL, DL, and ZM—were positive for the Y boundary, whereas the remainder—of which PP and LS are representative—were negative for the boundary. All XX males who had *ZFY* also had the Y boundary. Clinical details of the XX males will be published elsewhere[19].

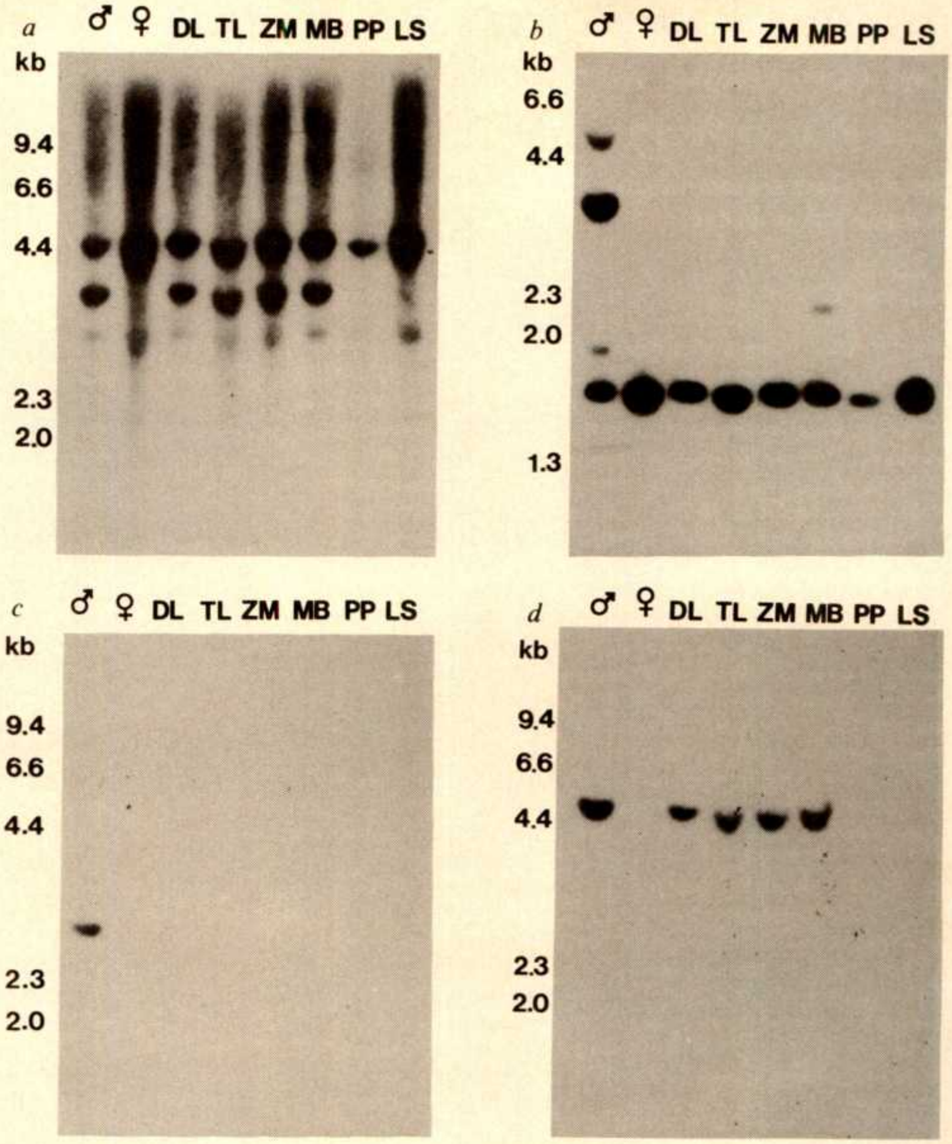

FIG. 3 Hybridization of probes to Southern blots. In each case filters contain the same lanes; ♂, normal male; ♀, normal female; DL, TL, ZM, MB, the four XX males discussed here, negative for *ZFY* but positive for the Y boundary; PP and LS, XX males negative for *ZFY* and the Y boundary. *a*, *Sac*I digests probed with Hf0.2—a pseudoautosomal probe[9]. *Sac*I gives a 4.5-kb X-specific fragment and a 3.2-kb Y-specific fragment across the pseudoautosomal boundary. *b*, *Eco*RI digests probed with a complementary DNA clone pMF-1, corresponding to a partial transcript of *ZFY* including the zinc-finger domain[6]. The three bands in the normal-male track not seen elsewhere are Y-specific. The weak 2.3-kb band seen in MB is probably due to a partial digestion and was not present in other digests of the same DNA sample in other experiments. *c*, *Hin*dIII digests probed with the Y-specific clone 27a[15]. Only the normal male was positive. *d*, *Eco*RI digests probed with the boundary proximal probe P0.9 (ref. 9). This probe is specific for the short arm of the Y chromosome.

somal boundary; this excludes *ZFY* as a candidate for *TDF*. These results also formally demonstrate that some XX males are not generated by terminal exchange and must be due to mutations elsewhere in the sex-determination pathway[10].

The only discrepancy between our results and those in previous publications is the description by Page *et al.*[4] of a female carrying a t(Y; 22) translocation which should include the Y pseudoautosomal boundary region. It is possible that this translocation is complex or, alternatively, *TDF* could have been inactivated by a position effect.

The four sex-reversed individuals vary in phenotype from normal male with testis, but lacking germ cells, to hermaphrodite with bilateral ovotestes and a uterus (Table 1). The variation in phenotype could be caused by the proximity of the breakpoints to *TDF*. Particularly striking are the two familial cases—the 'brother' and 'sister' TL and DL. Although the origin of these individuals is not clear, familial exchange XX males have been described previously[11], and one possibility is that the father has a mosaic gonad.

The results described here and by Koopman *et al.*[12] strongly argue against a role for *ZFY* in sex determination; *ZFY* could have a male specific function in spermatogenesis, however. Separate functions for *TDF* and *ZFY* have been proposed previously to explain the evolutionary relationship between the sex chromosomes of eutherians and metatherians[13], and could be consistent with the autosomal location of *ZFY*-related sequences in marsupials[8].

One explanation for the phenotypes we observe is that they result from the reciprocal breakpoints on the X chromosome. A more likely explanation is that the *TDF* gene lies close to, or even spans, the pseudoautosomal boundary. We are now testing these hypotheses. ☐

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1. Goodfellow, P. N. & Darling, S. M. *Development* **102,** 251–258 (1988).
2. Vergnaud, G. *et al. Am. J. hum. Genet.* **38,** 109–124 (1986).
3. Andersson, M., Page, D. C. & de la Chapelle, A. *Science* **233,** 786–788 (1986).
4. Page, D. C. *et al. Cell* **51,** 1091–1104 (1987).
5. Mardon, G. & Page, D. C. *Cell* **56,** 765–770 (1989).
6. Palmer, M. S., Berta, P., Sinclair, A. H., Pym, B. & Goodfellow, P. N. *Proc. natn Acad. Sci. U.S.A.* (in the press).
7. Schneider-Gädicke, A., Beer-Romero, P., Brown, L. G., Nussbaum, R. & Page, D. C. *Cell* **57,** 1247–1258 (1989).
8. Sinclair, A. H. *et al. Nature* **336,** 780–783 (1988).
9. Ellis, N. A. *et al. Nature* **337,** 81–84 (1989).
10. Petit, C. *et al. Cell* **49,** 595–602 (1987).
11. Page, D. C., de la Chapelle, A. & Weissenbach, J. L. *Nature* **315,** 224–226 (1985).
12. Koopman, P., Gubbay, J., Collignon, J. & Lovell-Badge, R. *Nature* (this issue).
13. Ellis, N. A. & Goodfellow, P. N. *Trends Genet.* (in the press).
14. Bengtsson, B. O. & Goodfellow, P. N. *Ann. hum. Genet.* **51,** 57–64 (1987).
15. Pritchard, C. A., Goodfellow, P. J. & Goodfellow, P. N. *Nucleic Acids Res.* **15,** 6159–6169 (1987).
16. Affara, N. A. *et al. Nucleic Acids Res.* **14,** 5375–5387 (1986).
17. Ellis, N. A. *et al. Nature* (submitted).
18. Goodfellow, P. N. *et al. Nature* **202,** 346–349 (1983).
19. Abbas, N. *et al. Hum. Genet.* (in the press).

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Zfy gene expression patterns are not compatible with a primary role in mouse sex determination

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THE Y chromosome determines maleness in mammals. A Y chromosome-linked gene diverts the indifferent embryonic gonad from the default ovarian pathway in favour of testis differentiation, initiating male development[1,2]. Study of this basic developmental switch requires the isolation of the testis-determining gene, termed *TDF* in humans and *Tdy* in mice. *ZFY*, a candidate gene for *TDF*, potentially encodes a zinc-finger protein[3], and has two Y-linked homologues, *Zfy-1* and *Zfy-2*, in mice[4,5]. Although *ZFY*, *Zfy-1* and *Zfy-2* seem to map to the sex-determining regions of the human and mouse Y chromosomes, there is no direct evidence that these genes are involved in testis determination. We report here that *Zfy-1* but not *Zfy-2* is expressed in differentiating embryonic mouse testes. Neither gene, however, is expressed in W^e/W^e mutant embryonic testes which lack germ cells. These observations exclude both *Zfy-1* and *Zfy-2* as candidates for the mouse testis-determining gene.

The primordial gonads (genital ridges) of male and female mouse embryos are indistinguishable until ~11.5 days post coitum (d.p.c.). By 12.5 d.p.c., testis differentiation is apparent from the presence of cords formed by the alignment of Sertoli cells. It is expected that the gene responsible for testis determination will be expressed around this stage of development. *Zfy-1* or *Zfy-2* expression is not detectable by northern blot analysis of RNA from whole mouse embryos[6]. To enrich for gonadal tissue, we isolated fetal genital ridges and gonads and analysed their RNA by northern blotting. *Zfy* transcripts were detected in adult testis but not in fetal gonads (Fig. 1*a*). By contrast, the X-linked homologue *Zfx* (ref. 5) was expressed at all stages. *Zfy* gene expression is clearly associated with germ cells in adult testes because transcripts were undetectable in XX *Sxr* and XX *Sxr'* testes which lack germ cells[7,8] (Fig. 1*b*).

Because of the relative insensitivity of northern blotting, we used the sensitive polymerase chain reaction (PCR) technique to look for *Zfy* transcripts in dissected embryonic tissues (Fig 2). Whereas no transcripts were detected in 9.5 d.p.c. embryos increasing levels of *Zfy-1* expression were seen between 10.5 and 12.5 d.p.c.; *Zfy-1* expression was maintained for at least two further days in the testes. Transcripts were absent from male nongonadal tissues. DNA blot analysis of the PCR products confirmed the onset of *Zfy-1* expression at 10.5 d.p.c. (Fig. 2*a*). The minor signal detected in the 12.5 d.p.c. ovary sample must reflect the occasional error in sexing embryos at this stage by gonadal morphology, given that RNA was derived from large numbers of pooled embryo samples. When RNA was prepared from genital ridges and gonads from individual embryos without pooling, a similar profile of *Zfy-1* expression between 10.5 and 14.5 d.p.c. was seen (Fig. 2*b*). In addition, decreasing levels of expression were found between 14.5 and 17.5 d.p.c.; all female samples were negative for *Zfy-1* expression.

By contrast, *Zfy-2* transcripts were detected in adult testes but not in embryonic mouse gonads at any of the stages examined (Fig. 2*a*). We conclude from this that *Zfy-2* is not *Tdy*.

The oligonucleotide primers used to detect *Zfy* transcripts were expected to give single PCR products of 477 base pairs (bp), based on published complementary DNA sequences[6,9]. Instead, two additional products of ~520 and 570 bp were detected (Fig. 2*b*). Sequencing of DNA from each band from 12.5-d.p.c. testes revealed three alternatively spliced *Zfy-1* transcripts (Fig. 3). No stage-specific difference in their relative abundance was seen. There is no obvious significance to this alternative splicing, which is within the 5′ untranslated portion of the messenger RNA. The additional exons in the longer *Zfy-1* transcripts do not introduce any alternative translational start sites associated with open reading frames, and would not alter the *Zfy-1* protein.

The embryonic gonad has both somatic and germ cell components, but it is clear that germ cells are not required for testis determination or differentiation[10,11]. The *W* mutation in mice affects primordial germ cell proliferation and migration into the genital ridge[12]; in embryos homozygous for the most severe form, W^e, testes form normally despite being virtually devoid of germ cells (ref. 13; and M. Buehr, personal communication).

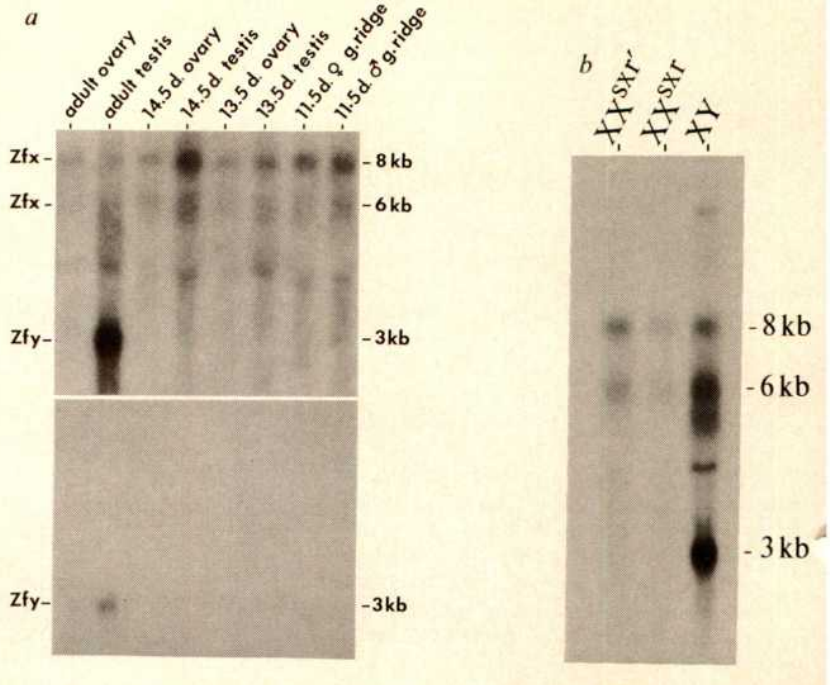

FIG. 1 Northern blot analysis of *Zfy* gene expression. *a*, Analysis of RNA from adult and embryonic mouse gonads and urogenital ridges (g. ridge) shows expression of a 3-kilobase (kb) *Zfy* transcript specific to adult testis. The upper panel shows hybridization to a 1.9-kb *Hin*dIII *Zfy-1* genomic fragment containing the zinc-finger encoding exon, which also detects 6- and 8-kb *Zfx* transcripts. The lower panel shows the same filter rehybridized with the first 800 bp of a *Zfy-1* cDNA[9] which is specific for *Zfy* sequences. *b*, Absence of the 3-kb *Zfy* transcript in XX *Sxr* and XX *Sxr'* adult testes which lack germ cells. The 'zinc-finger' probe described above shows that *Zfx* transcripts are present in both testis types as well as in normal XY testes.

METHODS. Total RNA (10 μg) was electrophoresed on formaldehyde-agarose and transferred to Hybond-N filters (Amersham). Filters were hybridized to ^{32}P-labelled random-primed DNA probes, then washed in 0.1 × SSC-0.1% SDS at 50 °C for 1 h and exposed to X-ray film for 1-3 days. Parkes outbred mice were used for all experiments unless otherwise stated. The day of vaginal plug was taken to be 0.5 d.p.c. Embryos 9.5-11.5 d.p.c. were sexed by staining for sex chromatin in amnion cells[17], and thereafter by gonadal morphology.

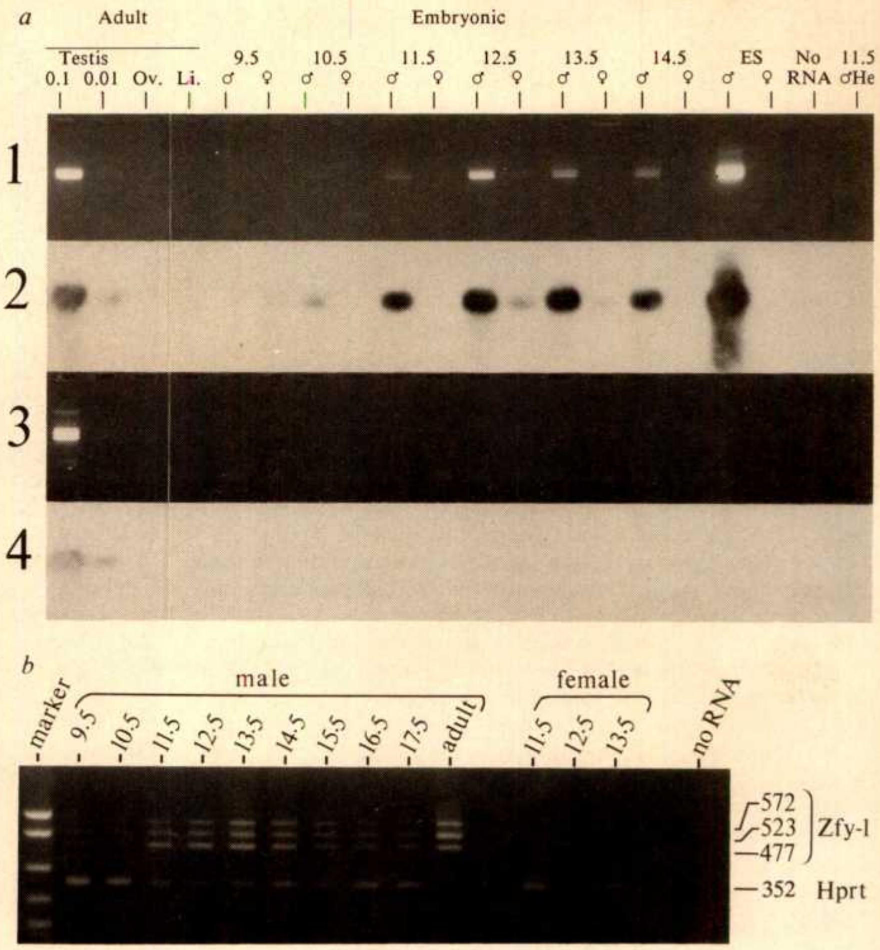

FIG. 2 PCR analysis of *Zfy-1* and *Zfy-2* expression in embryonic mouse gonads. *a*, Bands correspond to *Zfy-1* and *Zfy-2* transcripts in RNA derived from tissue pooled from up to 200 embryos or adults. Rows 1 and 3: ethidium bromide-stained agarose gels showing PCR products obtained using primers specific for *Zfy-1* and *Zfy-2*, respectively. Rows 2 and 4: corresponding Southern blots hybridized with the *Zfy*-specific probe described in Fig. 1 legend. These gels did not allow resolution of the three separate bands associated with *Zfy* expression (see below and Fig. 3). *b*, PCR analysis using RNA prepared from individual embryos or from adult testis. Ethidium bromide-stained agarose gel showing three PCR products obtained using *Zfy-1*-specific primers; specific primers for hypoxanthine phophoribosyl transferase (Hprt) transcripts were included as a positive control for each sample. Band sizes are shown in base pairs. Abbreviations: Ov, adult ovary; Li, adult male liver; 9.5, 9.5-d.p.c. embryos posterior to forelimb bud; 10.5, 10.5-d.p.c. embryos between forelimb and hindlimb buds; 11.5, 11.5-d.p.c. urogenital ridges; 12.5–17.5, 12.5–17.5-d.p.c. fetal gonads; ES, embryonic stem cells, either CCE[18] (♂) or A13[19] (♀); No RNA, water added to reverse transcription reactions in place of RNA solution; 11.5♂He, heads of 11.5-d.p.c. male embryos; Marker, pBR322/*Msp*I fragments (622, 527, 404, 309 and 242 bp).

METHODS. *a*, Total RNA (1 μg; 0.1 or 0.01 μg from adult testis) was reverse transcribed in 30 μl as described in ref. 5, at 42 °C for 30 min. Of this, 5 μl was added to a 50-μl PCR reaction containing 0.05% Nonidet P-40, 1.5 mM each dNTP, 500 ng each 'outer' primer, and 2.5 U *Taq* polymerase in the supplied buffer (Anglian Biotec). DNA was amplified by 20 cycles of 94 °C for 5 s, 65 °C for 30 s, and 72 °C for 30 s in a Techne PHC-2. From each reaction, 1 μl was transferred to a new reaction containing 'nested' primers and amplified a further 20 cycles. A 5 μl aliquot was electrophoresed on a 2% agarose–TBE gel. *b*, Small-scale RNA preparations were made using the AGPC method[20]. The amount of RNA reverse-transcribed corresponded to 100% of 9.5 and 10.5-d.p.c. tissue, 100% of a single 11.5-d.p.c. urogenital ridge or 12.5-d.p.c. gonad, 40%, 20%, 12.5%, 9%, and 7.6% of single testes from 13.5 to 17.5 d.p.c., respectively, 50% of a 13.5-d.p.c. ovary, and 0.5% of an adult testis. These proportions were intended to reflect gonadal volume. In each case the entire 7.5-μl reverse transcription reaction was amplified as above, with the addition of 500 ng of each *Hprt* primer to the first PCR reaction; 20 μl of the second reaction was electrophoresed. Primer sequences (5′–3′) were as follows. *Zfy-1* outer primers were (5′) GTTAC TCATT TTCAG GTGTT CTGGG and (3′) GTGTC AGCTG TTATA GGATC AGTGA; *Zfy-2* outer primers were (5′) CCTGT TGTTG CTGTT GTGGT TCTCG and (3′) ATGTC GGCTG TCAAA GGATC AGTGG. These primers were from corresponding regions of the *Zfy-1* and *-2* cDNAs[6,9]. Nested primers are described in Fig. 3 legend. *Hprt* primers were (5′) CCTGC TGGAT TACAT TAAAG CACTG and (3′) GTCAA GGGCA TATCC AACAA CAAAC from exons 3 and 8 of the mouse gene, respectively[21].

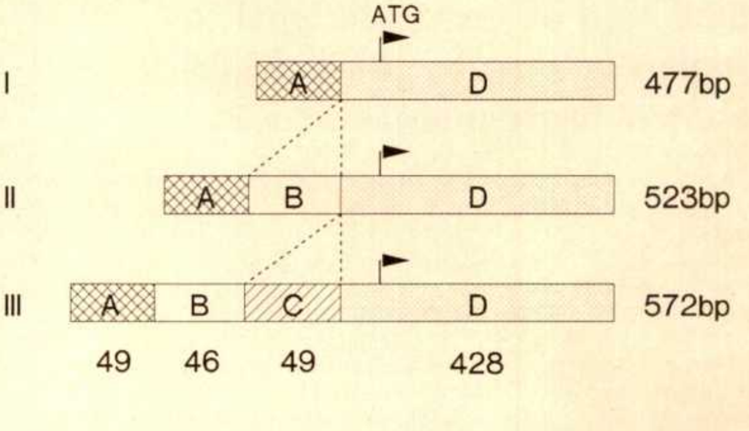

FIG. 3 Alternative splicing of *Zfy-1* transcripts. The composition of the three different PCR products obtained from 12.5-d.p.c. testis RNA with the *Zfy-1* primers was deduced from DNA sequencing. The nucleotide sequence and size of each block is shown; the translation initiation codon ATG is indicated in block D. Transcript I corresponds to the published *Zfy-1* cDNA sequence[9], except for the single base difference shown in lower case, presumably a polymorphism between the *Mus musculus musculus* and *M. m. domesticus* subspecies. Transcripts II and III contain the additional exons B and C.

METHODS. PCR as described in Fig. 2 legend; nested primer sequences are shown underlined. DNA from each gel band was cloned into Bluescript (Stratagene) and six clones of each type sequenced using Sequenase (USB).

A. TGAAGTCTGCAGTACTTGTCGTCATTGCCTACCCCTCTACACTGGTCTG

B. ATTATCTGAATAATGTTATAATATGTTCCTGACATCATTCTGTTCT

C. ACATGAATTTATATTTATTATACCTGCTATCAGTTCATTTATGGCCCAG

D. GAGCTGACTTAGTAAGAACTGAAGGCC ATG GATGAAGATGAAATT
GAATtGACC.............TGCCTGAACATGTCTTGATGAGTGA

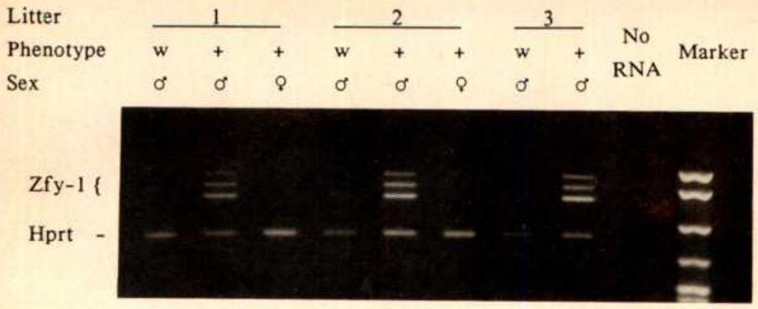

FIG. 4 Lack of *Zfy-1* expression in W^e/W^e fetal testes. The three bands corresponding to *Zfy-1* expression were detectec using RNA from phenotypically wild-type (+) male fetal testes but not phenotypically mutant (w) testes. The control *Hpr* band was detected in all samples. Littermates from three separate litters were analysed.

METHODS. Random-bred C3H/H-101 W^e/+ mice were matec and the progeny scored as homozygous mutants by liver morphology at 14.5 d.p.c., (ref. 22) and sexed by gonad morphology. Small-scale RNA preparations were made and analysed as described in Fig. 2 legend. In the first round of the PCR, 50% of the RNA yield from each single gonad was used.

Further, data from XX↔XY chimaeras implies that *Tdy* must act cell-autonomously in the somatic supporting cell lineage of the genital ridge[14]. If *Zfy-1* is indeed *Tdy*, expected transcripts of *Zfy-1* should be found in the somatic portion of the developing testis.

To test this, we analysed embryos homozygous for the W^e mutation. Their testes lacked *Zfy-1* transcripts (Fig. 4), demonstrating that *Zfy-1* expression in normal testes is due to the presence of germ cells. An alternative pair of PCR primers, from the exons encoding the putative nuclear localization signal and the 'zinc-finger' motifs[6,9], confirmed the absence of *Zfy-1* and *Zfy-2* transcripts in W^e/W^e fetal testes (data not shown). The formation of testes in the absence of *Zfy* expression, and the association of *Zfy-1* expression with germ cells, argues that *Zfy-1* is not *Tdy*.

ZFY was initially regarded as a candidate for *TDF* by virtue of its location on the Y chromosome and because its putative product is consistent with cell-autonomous action. Several more recent observations have compromised this view. First, theories of sex determination involving *ZFY*-like genes must take into account the presence of an X-linked homologue in many species and an additional autosomal homologue in mice[3,5]. Second, *ZFY*-like sequences are not found on the sex chromosomes of marsupials, which seem also to have a Y-dependent sex-determining mechanism[15]. Third, *Zfy-1* and *Zfy-2* appear to be expressed normally in a line of mice shown genetically to be mutant in *Tdy* (R.L.-B *et al.*, manuscript in preparation). Fourth, XX male patients have been described who possess Y-linked sequences but not *ZFY*[16]. Finally, the sites of expression of *Zfy-1* and *Zfy-2* indicate that neither is *Tdy*, and that both may instead have a role in male germ cell development. □

Received 14 November; accepted 23 November 1989.

1. McLaren, A. *Trends Genet.* **4,** 153–157 (1988).
2. Goodfellow, P. N. & Darling, S. M. *Development* **102,** 251–258 (1988).
3. Page, D. C. *et al. Cell* **51,** 1091–1104 (1987).
4. Mardon, G. *et al. Science* **243,** 78–80 (1989).
5. Nagamine, C. M., Chan, K., Kozak, C. A. & Lau, Y.-F. *Science* **243,** 80–83 (1989).
6. Mardon, G. & Page, D. C. *Cell* **56,** 765–770 (1989).
7. Cattanach, B. M., Pollard, C. E. & Hawkes, S. G. *Cytogenetics* **10,** 318–337 (1971).
8. Burgoyne, P. S., Levy, E. R. & McLaren, A. *Nature* **320,** 170–172 (1986).
9. Ashworth, A., Swift, S. & Affara, N. *Nucleic Acids Res.* **17,** 2864 (1989).
10. Merchant, H. *Devl Biol.* **44,** 1–21 (1975).
11. McLaren, A. in *The Origin and Evolution of Sex* (eds Halvorson H. O. & Monroy, A.) 289–300 (Liss, New York, 1985).
12. Mintz, B. & Russell, E. S. *J. exp. Zool.* **134,** 207–237 (1957).
13. Cattanach, B. M. *Mouse News Lett.* **59,** 18 (1978).
14. Burgoyne, P. S., Buehr, M., Koopman, P., Rossant, J. & McLaren, A. *Development* **102,** 443–450 (1988).
15. Sinclair, A. H. *et al. Nature* **336,** 780–783 (1988).
16. Palmer, M. S. *et al. Nature* **342, ■■■–■■■** (1989).
17. Monk, M. & McLaren, A. *J. Embryol. exp. Morph.* **63,** 75–84 (1981).
18. Robertson, E., Bradley, A., Kuehn, M. & Evans, M. *Nature* **323,** 445–448 (1986).
19. Robertson, E. J., Kaufman, M. H., Bradley, A. & Evans, M. in *Cold Spring Harbor Conferences on Cell Proliferation, Vol. 10: Teratocarcinomas and Stem Cells* (eds. Silver, L. M., Martin, G. R. & Strickland, S. 647–663 (Cold Spring Harbor Lab. New York, 1983).
20. Chomczynski, P. & Sacchi, N. *Analyt. Biochem.* **162,** 156–159 (1987).
21. Melton, D. W., Konecki, D. S., Brennand, J. & Caskey, C. T. *Proc. natn. Acad. Sci. U.S.A.* **81,** 2147–2151 (1984).
22. Borghese, E. *Acta Anat.* **36,** 185–220 (1959).

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Inhibition of endocytic vesicle fusion *in vitro* by the cell-cycle control protein kinase cdc2

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MEMBRANE transport between the endoplasmic reticulum and the plasma membrane, which involves the budding and fusion of carrier vesicles, is inhibited during mitosis in animal cells[1–5]. At the same time, the Golgi complex[6] and the nuclear envelope[7], as well as the endoplasmic reticulum in some cell types, become fragmented. Fragmentation of the Golgi is believed to facilitate its equal partitioning between daughter cells[8]. In fact, it has been postulated that both the inhibition of membrane traffic and Golgi fragmentation during mitosis are due to an inhibition of vesicle fusion, while vesicle budding continues[8]. Although less is known about the endocytic pathway, internalization[9–11] and receptor recycling[12,13] are also arrested during mitosis. We have now used a cell-free assay to show that the fusion of endocytic vesicles from baby hamster kidney cells is reduced in *Xenopus* mitotic cytosol when compared with interphase cytosol. We reconstituted this inhibition in interphase cytosol by adding a preparation enriched in the starfish homologue of the cdc2 protein kinase. Inhibition was ≥90% when the added cdc2 activity was in the range estimated for that in mitotic *Xenopus* eggs, which indicates that during mitosis the cdc2 kinase mediates an inhibition of endocytic vesicle fusion, and possibly other fusion events in membrane traffic.

To study the regulation of membrane traffic in mitotic cells, we used a cell-free assay that reconstitutes the fusion of early endocytic vesicles. In interphase cells, this fusion apparently reflects lateral interactions between early endosomal elements (for a review, see ref. 14). This fusion may also share an *N*-ethylmaleimide-sensitive factor[15] and the involvement of GTP-binding proteins[16] with vesicular transport in the secretory pathway[17–19].

As in our previous studies[20], the early endocytic vesicles of two baby hamster kidney (BHK) cell populations were labelled separately with avidin and biotinylated horseradish peroxidase (bHRP) by fluid-phase endocytosis for 5 min at 37 °C. Cytosol-free endosomal fractions were then prepared from each cell population, mixed in the fusion assay with cytosol and incubated

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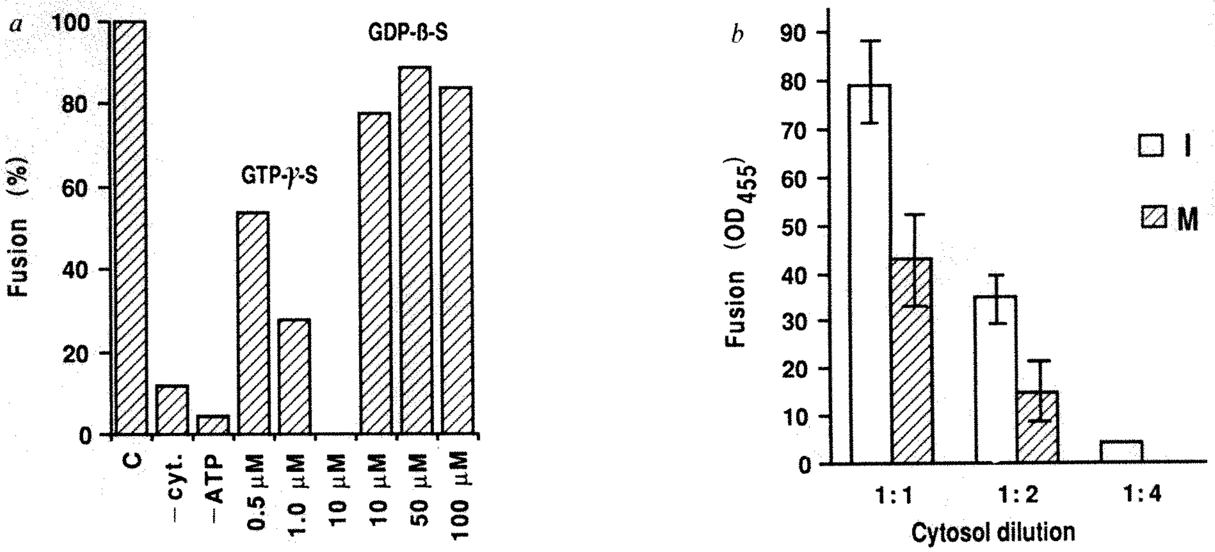


FIG. 1 Early endocytic vesicle fusion reconstituted in *Xenopus* interphase and mitotic cytosols. *a*, To compare different experiments, fusion is expressed as a percentage of the control value. C, control; −cyt., without cytosol; −ATP, without ATP. Concentrations of GTP-γ-S and GDP-β-S as indicated.

METHODS. BHK cells from 15×75-cm^2 dishes were released with trypsin, diluted three times with ice-cold PBS containing 5 mg ml^{-1} BSA (PBS-BSA), centrifuged at 750*g* for 5 min and washed a further three times in ice-cold PBS-BSA. The cells were then incubated for 5 min at 37 °C with 3.3 mg ml^{-1} avidin or 1.7 mg ml^{-1} bHRP in medium[20] and washed three times by centrifugation as above. The cells were subsequently resuspended in 10 ml sucrose (0.25 M), 3 mM imidazole pH 7.4 (HB), centrifuged, resuspended in 1 ml HB and homogenized in a tight-fitting Dounce. A post-nuclear supernatant was prepared by centrifugation at 1500*g* for 15 min, made up to a concentration of 40.6% sucrose, 0.5 mM EDTA in 3 mM imidazole pH 7.4, and loaded in 0.4-ml aliquots at the bottom of a Beckman polycarbonate 11×34-mm tube. The tube was then filled sequentially with a cushion of 0.6 ml sucrose (35%), 3 mM imidazole pH 7.4, 0.5 mM EDTA and 0.3 ml HB, 0.5 mM EDTA. The avidin- (or bHRP-) containing early endocytic vesicles were floated to the 35% sucrose-HB interface by centrifugation for 45 min at 100,000*g* in a table-top TL-100 ultracentrifuge, using a TLS-55 rotor. The interface fraction was collected with a pump, frozen in 25-μg aliquots in liquid nitrogen and stored until use. Concentrated *Xenopus* interphase and mitotic extracts were prepared as in ref. 25, and contained ~40 mg protein per ml. In the fusion assay, 25 μg of each fraction (avidin and bHRP) were mixed on ice with 50 μl cytosol (~2 mg protein). The volume of the reaction was 150 μl and the mixture contained 50 mM potassium acetate, 12.5 mM HEPES buffer, 1 mM magnesium acetate, 1.5 mM dethiothreitol (DTT), 1 μg ml^{-1} cytochalasin D and an ATP-regenerating or -depleting system as described in ref. 20. The fusion reaction was carried out for 45 min at 37 °C in the presence of biotinylated insulin, the samples were then returned to ice-temperature, excess biotinylated insulin added and the samples extracted in detergents[20]. The fusion-specific complex (avidin-bHRP) was quantitated with ELISA by measuring the enzymatic activity of bHRP at 455 nm (ref. 20). For *b*, The experiment was as for *a*, except that 50 μl (1:1), 25 μl (1:2) and 12.5 μl (1:4) interphase (I) and mitotic (M) extracts were added to the reaction mixture, which was adjusted to 150 μl, and to the same concentration of reagents as in Fig. 1*a*. The enzymatic activities of bHRP (optical density (OD) at 455 nm $\times10^3$) complexed to avidin are indicated for the different extracts.

in vitro for 45 min at 37 °C. During the *in vitro* reaction, fusion between avidin- and bHRP-containing endocytic vesicles resulted in the formation of an avidin-bHRP complex. At the end of the reaction, the mixture was extracted in detergents in the presence of excess biotinylated insulin to quench the uncomplexed avidin, and the amount of avidin-bHRP formed was quantitated with enzyme-linked immunosorbent assay (ELISA)[20,21]. In the studies described here, it was important to use a well-characterized source of mitotic and interphase cytosols. Concentrated extracts (~40 mg per ml protein) were prepared by high-speed centrifugation of *Xenopus* eggs that were either arrested in metaphase II[22] or activated by an electric shock[23]. The mitotic status of the extracts was estimated by measuring H1 histone kinase activity, and corresponded to 0.5–1.0 pmol of phosphate transferred from ATP to H1 histones min^{-1} μl^{-1} and >8.0 pmol of phosphate transferred from ATP to H1 histones min^{-1} μl^{-1} for interphase and mitotic extracts, respectively. These activities were expected to reflect the cytosolic values *in vivo*[24], because the extracts were prepared after a minimal dilution of the egg volume (30% v/v dilution). Fusion of BHK endocytic vesicles could be reconstituted in interphase *Xenopus* cytosol and, as expected, required cytosol as well as ATP (Fig. 1*a*). The extent of fusion depended on the concentration of added cytosol (Fig. 1*b*). Specificity of the fusion reaction was shown by its inhibition at low GTP-γ-S concentrations (Fig. 1*a*). This observation and others[16] indicate that GTP-binding proteins may be involved in controlling endocytic

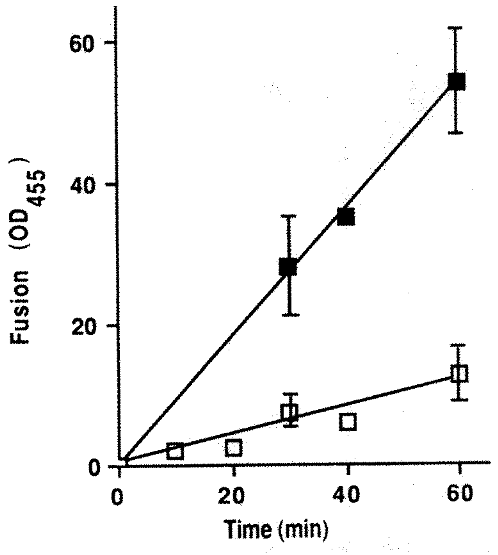


FIG. 2 Time course of the fusion with (□) or without (interphase control, ■) cdc2 kinase. The experiment was as in Fig. 1*a*, except that 1 μl cdc2 kinase prepared as in ref. 24 up to the phosphocellulose step, was added to each fraction and to an aliquot of interphase cytosol. For maximal inhibition, each fraction and the cytosol were pre-incubated separately for 15 min at 22 °C in the presence of cdc2 kinase, mixed and then further incubated at 37 °C for the indicated times to allow fusion to proceed. Fusion was measured by bHRP activity at OD_{455} and is indicated as in Fig. 1*b*.

vesicle fusion. By contrast, high concentrations of GDP-β-S had little effect.

When the assay was carried out in the presence of mitotic cytosol, the fusion activity was reduced (Fig. 1*b*) and the inhibition observed with different extracts paralleled the H1 histone kinase activities measured during the reaction (see below). This prompted us to test purified preparations of the kinase in the fusion assay. In mitotic *Xenopus* extracts, most of the H1 histone kinase activity is due to the homologue of the fission yeast $p34^{cdc2}$ and the cdc2 kinase from mitotic yeast phosphorylates H1 histones[26]. The role of the cdc2 kinase in controlling the interphase-mitosis transition in yeast, as in animal cells, is now well established[27,28]. Using *in vitro* assays, the maturation promoting factor (MPF), which has cdc2 activity, was shown to mediate nuclear envelope breakdown[29-31], chromosome condensation and spindle assembly[30,31].

Partially purified preparations of cdc2 kinase were obtained from starfish with high activities corresponding to the transfer from ATP to H1 histone of ~800 pmol phosphate per min per µl extract. These cdc2 kinase preparations were used to complement the fusion assay in the presence of interphase cytosol. In the absence of cdc2 kinase, fusion increased linearly with the time of the reaction (Fig. 2). When the cdc2 kinase was added at an activity corresponding to 4-5 pmol phosphate transferred per min per µl reaction mixture, a 75% reduction in the extent of fusion was observed for up to 60 min. Activity of the cdc2 kinase in the assay was also tested using gel electrophoresis and autoradiography by following the phosphorylation of endogenous substrates present in the reaction mixture after 5-min pulses of $[^{32}\mathrm{P}]$ATP (data not shown).

To establish the relationship between the cdc2 kinase activity and the inhibition of fusion, the assay was performed in the presence of increasing amounts of cdc2 kinase (Fig. 3). This titration correlated well with individual observations made with two different preparations of the cdc2 kinase (Fig. 3). Moreover, the inhibitions observed with mitotic cytosols (see Fig. 1*b*) were well within the range expected from the H1 histone kinase activities of the corresponding reaction mixtures (Fig. 3). This correlation strongly indicates that inhibition of fusion was mediated by the cdc2 kinase. Fusion activity rapidly decreased as the kinase activity became greater than that corresponding to the transfer of 1.0 pmol phosphate to H1 histones per min per µl reaction mixture. Half-maximal inhibition was observed at an activity corresponding to the transfer of 2.3 pmol phosphate per min per µl reaction mixture, and fusion was essentially abolished at activities corresponding to the transfer of ≥10 pmol phosphate per min per µl reaction mixture. Because the kinase activity of the cytosol in mitotic *Xenopus* eggs is estimated to be in this latter range, early endocytic vesicle fusion *in vivo* is probably efficiently arrested during mitosis.

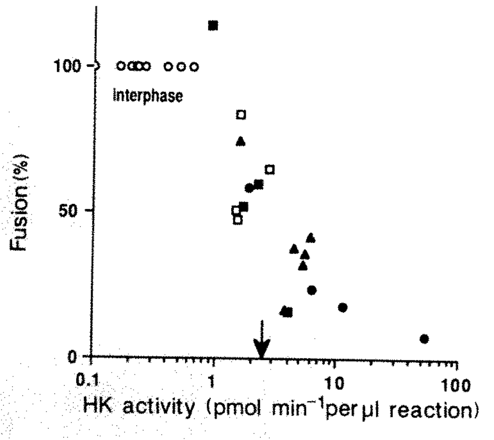


FIG. 3 Titration of fusion at increasing cdc2 kinase activity. Fusion activity is expressed as a percentage of the interphase value so that comparison between different experiments can be made. Titration of the fusion at increasing cdc2 activity (●) is compared with individual experiments carried out with two preparations of cdc2 kinase as in Fig. 2 (■, ▲). □, Assays using mitotic extracts as in Fig. 1*b*. The H1 histone kinase (HK) activities of the latter reaction mixtures were lower than those of the original mitotic extracts partly because the extracts were diluted three times in the final volume of the reaction (see Fig. 1*a*). The HK activity was measured as in ref. 25.

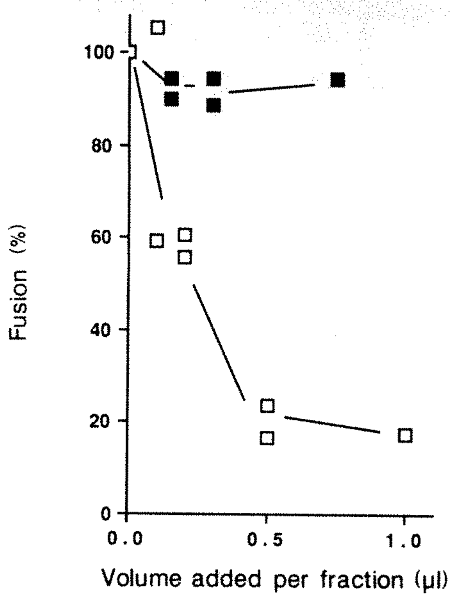


FIG. 4 Depletion of $p34^{cdc2}$ with $p13^{suc1}$ beads. The experiments were carried out as in Fig. 3 after adding the indicated volumes of the cdc2 preparation before (□) or after (■) $p34^{cdc2}$ depletion. Specific and quantitative depletion of the $p34^{cdc2}$ was carried out by affinity chromatography using $p13^{suc1}$ as a ligand[34]. The $p34^{cdc2}$-depleted preparations were used in the assay at the same protein concentration as the original $p34^{cdc2}$-containing preparations.

To demonstrate further that fusion inhibition was caused by the cdc2 kinase, the purified starfish preparation was depleted of $p34^{cdc2}$ by affinity-binding to the product of the fission yeast *suc1*⁺, gene $p13^{suc1}$, coupled to agarose beads. Depletion was efficient and specific, as monitored by H1 histone kinase activity and western blotting with an antibody against the highly conserved PSTAIR amino-acid sequence of $p34^{cdc2}$ (data not shown). The association of $p34^{cdc2}$ with $p13^{suc1}$ (refs 32, 33) has been used to retrieve $p34^{cdc2}$ (refs 34-36). Figure 4 shows that fusion was not inhibited after $p34^{cdc2}$ depletion. Finally, $p34^{cdc2}$ retained on $p13^{suc1}$ beads could be eluted with excess $p13^{suc1}$ and further purified by an additional chromatography step on a mono-S column in a 1:1 stoichiometric complex between $p34^{cdc2}$ and cyclin B (ref. 34). After addition of affinity-purified $p34^{cdc2}$, a 40% inhibition was observed when the histone kinase activity measured in the assay corresponded to the transfer of 1.5 pmol phosphate per min per µl. These values agree well with the titration presented in Fig. 3.

These *in vitro* findings strongly indicate that early endocytic vesicle fusion is arrested during mitosis and that this arrest is mediated by the cell-cycle control protein kinase cdc2. Although not directly shown here, this inhibition is expected to be the result of the protein-kinase activity of cdc2. Whether other fusion events are similarly inhibited in mitotic cells, accounting for the observed arrest of intracellular transport, remains to be determined. It is tempting to postulate that cdc2 kinase is part of the central regulatory mechanism controlling membrane traffic in mitotic cells. □

activation of *Prl* may require synergistic interactions with the enhancer region. Similar results were observed in transgenic mice[6].

To determine the structural features of GHF-1 responsible for its activity as a transcription factor, a series of internal deletion mutants was generated (Fig. 2). The ability of these mutants to activate the GH gene promoter was examined by a co-transfection assay in both F9 and Rat-6 cells. Although the extent of GH gene promoter activation in this assay was not as high as that observed in HeLa *MT-GHF1*$^+$ cells, because of a higher basal level, it was more convenient for screening a large number of mutant proteins. In this assay, activation of the GH gene promoter was also dependent on the presence of GHF-1-binding sites (data not shown).

Three different regions are required for efficient activation of *GH-CAT* by GHF-1. Deletion of amino-acid residues 2-45 and 48-73, within a serine-threonine-rich region, led to a large decrease in activity. Similarly, deletion of residues 124-201 encompassing the POU-specific domain, and residues 209-252 and 255-291 within the homoeodomain, had a strong deleterious effect. By contrast, deletion of residues 72-125 containing a cluster of negatively charged residues, and residues 200-211 constituting a putative hinge region between the POU-specific domain and the homoeodomain, did not impair activity. In fact, GHF-1 with a deletion of residues 72-125 (mutant Δ72-125) was more active than wild-type GHF-1. All of the mutants, with the exception of Δ125-201, which could not be tested because of the loss of the antigenic epitope, and Δ211-254, were efficiently expressed and accumulated in the nucleus (Fig. 2). Mutant Δ211-254 lacking the N-terminal half of the homoeodomain exhibited diffuse cytoplasmic fluorescence (data not shown) indicating that the nuclear translocation signal of GHF-1 is located within the deleted region.

The homoeodomain is the minimal DNA-binding domain of several proteins[14-16]. To analyse the DNA-binding domain of GHF-1, two chimaeric proteins were constructed, Jun-G123 and Jun-G202, by fusing the activation domain of c-Jun[17] to C-terminal GHF-1 sequences either containing the POU-specific domain (residues 123-291) or lacking the POU-specific domain (residues 202-291). Both chimaeras specifically activated *GH-CAT* expression; the one containing the POU-specific domain was fivefold more active than the one that lacked this region (Fig. 3*a*). The role of the POU-specific domain in DNA binding was also investigated by preparing two TrpE-GHF-1 fusion proteins. One, TrpE-GHF-1, contained the entire GHF-1 sequence (residues 1-291), whereas the other, TrpE-GHF(HB), contained the entire homoeodomain and only the C-terminal half of the POU-specific domain (residues 164-291; Fig. 3*b*). Footprinting (Fig. 3*c*) and mobility-shift (data not shown) experiments indicated that the protein containing the entire POU-specific domain exhibits 2.5- to 3-fold higher relative affinity than the protein lacking half of this motif. Most importantly, binding of the two proteins resulted in very similar DNase I protection patterns. As shown previously[8], these footprints were essentially identical to those generated by native GHF-1 purified from pituitary cells.

Although TrpE-GHF(HB) also lacks the N-terminal half of the protein, taken collectively, these results strongly indicate that the POU-specific domain of GHF-1 is not directly involved in making contact with the DNA, but somehow potentiates binding by the homoeodomain, which, as in other members of this family[14-16], is the minimal DNA-binding domain. It is possible that the POU-specific domain is responsible for increasing the binding specificity of GHF-1, because high concentrations of TrpE-GHF(HB) protect additional sequences not recognized by native GHF-1 (ref. 8). One possible explanation for the difference between our results and those of Sturm and Herr[18] is that, unlike GHF-1, Oct-1 could be more dependent on the POU-specific domain because of the lower intrinsic DNA-binding affinity of its homoeodomain.

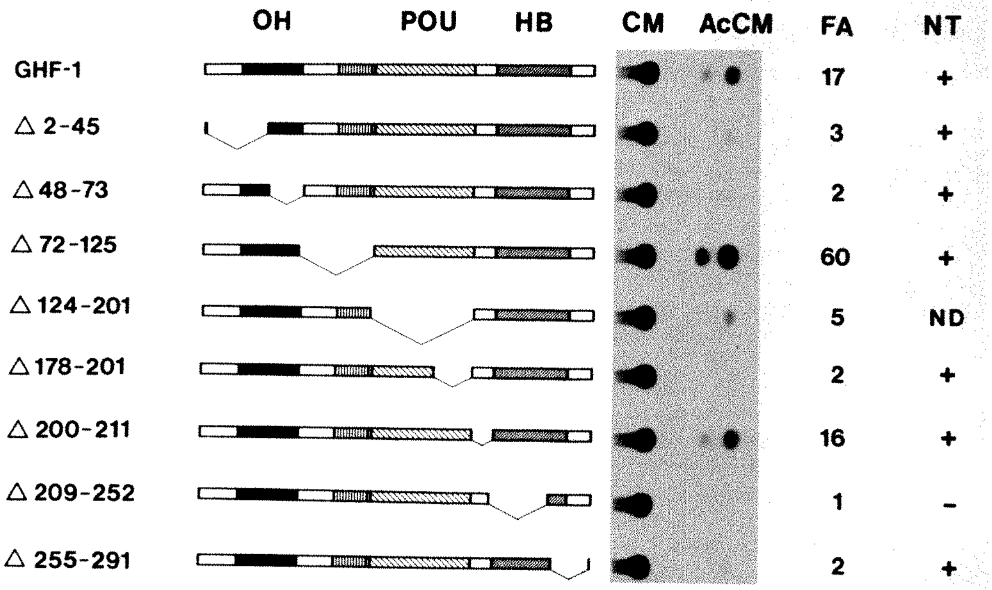


FIG. 2 Mutational analysis of GHF-1. The structure of GHF-1 and the internal deletion mutants are shown in a schematic form. Hatched boxes, homoeodomains (HB) and POU-specific domains (POU); striped boxes, regions rich in negatively charged residues; solid black boxes, regions rich in hydroxylated amino acids (OH); and open boxes, remainder of protein. Thin lines denote deleted sequences. Mutants are named according to the sequences affected by the deletions. The activities of the various constructs were tested by co-transfection with the *GH-CAT* reporter gene and determination of CAT activity. Results of one typical assay using Rat-6 cells are shown next to the diagram outlining the various constructs. Abbreviations: CM, chloramphenicol; acCm, acetylated chloroamphenicol. Fold activation (FA) is the activity of the various constructs relative to that of Δ209-252 which does not activate the GH gene promoter. Similar results were seen in two other assays in both Rat-6 and F9 cells. No activation of the construct containing the mutant GH gene promoter, *GH*Δ*(−82/−128)-CAT* (Fig. 1) was observed (data not shown). Nuclear transfer (NT) of the various proteins was determined by indirect immunofluorescence using an antibody specific for GHF-1. ND, not detected. These assays also served as controls for the quality of the transfections. Levels of GHF-1 expression were similar for all of the constructs indicated as NT positive (+).

METHODS. The *RSV-GHF-1* expression vector described in Fig. 1 was used as a template for generating by PCR[33] the various deletion mutants. Mutagenic primers were complementary to the sequences flanking the deleted regions such that one series of reactions generated 5′ fragments and the other 3′ fragments. These fragments were joined together to yield the final constructs, whose identity was confirmed by sequencing[34]. *GH-CAT* (5 μg) were transfected into Rat-6 or F9 cells on 10-cm plates together with 10 μg of the various expression vectors and 5 μg of carrier DNA, and CAT activity was determined 40 h later. Similar results were obtained in both F9 and Rat-6 cells. For immunofluorescence analysis, F9 cells were grown on cover slides and transfected as described above. Cells were fixed 10 h after removal of DNA precipitate and examined by indirect immunofluorescence with anti-GHF-1 antibodies[8].

Because the N-terminal half of GHF-1 is not involved in DNA binding, deletions of residues 2-45 and 48-73 probably affect the transcriptional activation domain. It is of interest that residues 1-73 contain only three sparsely spaced negatively charged residues, whereas residues 72-125, the deletion of which did not impair function, contain most (10 out of 13) of the negatively charged residues within the N-terminal domain. Therefore, residues 1-73 could constitute a novel activation domain that is different from the well-characterized negatively charged activation domains found in a variety of yeast and mammalian proteins[17,19-21]. Fusion of different portions of GHF-1 to the c-Jun DNA-binding domain, which recognizes the AP-1 binding site[17,22], confirmed these assertions. Despite possible interactions with endogenous Jun and Fos proteins, the c-Jun DNA-binding domain of the mutant protein JunΔ6-223 is practically inactive[17]. Fusion of GHF-1 residues 1-71 was sufficient to confer transcriptional stimulatory activity on the c-Jun DNA-binding domain (Fig. 4*a*). These experiments also indicate that residues 71-208 contribute very little to this activity. The primary sequence of the GHF-1 activation domain is shown in Fig. 4*b*.

The results described above demonstrate that the pituitary-specific transcription factor, GHF-1, is composed of two functional domains. The C-terminal half of this protein of relative molecular mass 33,000 (M_r 33K) contains its DNA-binding domain, whereas the N-terminal half is involved in *trans*-activation. Like other homoeodomain proteins[14-16], the minimal DNA-binding domain of GHF-1 is composed of its homoeodomain. The N-terminal portion of the homoeodomain is also likely to contain the nuclear transfer signal, a good candidate for which is a stretch of six contiguous basic residues between positions 213 and 219, similar to other nuclear transfer signals[23]. In addition to the homoeodomain, optimal DNA-binding requires the POU-specific domain. But, the exact part played by this motif is not clear because it does not seem to be involved in making contact with the specific binding sites for GHF-1. It is possible that the POU-specific domain stabilizes the protein–DNA complex and increases binding specificity by a so far unknown mechanism.

The N-terminal half of GHF-1 is responsible for *trans* activation. Construction of GHF-1–Jun chimaeric proteins enabled the demonstration that the first 71 amino-acid residues of GHF-1 function as an independent activation domain. This part of the protein contains only three sparsely spaced negatively charged residues and two glutamines (Fig. 4*b*). Therefore it bears no primary sequence homology to the two prototypes of activation domains described previously[19-21,24]. By contrast, more than 30% of the amino and residues in this region of GHF-1 are hydroxylated residues: serine, threonine and tyrosine. Because of phosphorylation, this region could constitute an acidic activator[21]. But immunoprecipitation of ^{35}S- and ^{32}P-labelled GHF-1 indicates that the extent of GHF-1 phosphorylation is very low (L. H. K. Defize, unpublished results). In addition, competition experiments indicate that the GHF-1 fails to compete against the acidic activation domain of c-Jun, whereas homologous competition is very efficient (unpublished results). We conclude therefore that the activation domain of GHF-1 represents a new class of activation domains.

So far the essential structural features of this domain are not known. The most remarkable feature of this part of GHF-1, however, is the abundance of hydroxylated amino acids. (Fig. 4*b*) and therefore, we have named it the STA domain (Ser-Thr-rich activation domain). It is noteworthy that two other members of the POU-domain family, Oct-2 and Unc-86, both of which are cell type-specific activators, contain regions that are abundant (>30%) in hydroxylated amino acids. In Oct-2 this region is located near its C terminus[25-27], and in Unc-86 it is located immediately upstream of the POU-domain[28]. Two other cell type-specific *trans*-acting factors that do not belong to the homoeodomain family, MyoDl and Myf-5, also contain Ser-Thr-rich regions[29]. At least for Oct-2, this region could be important for *trans*-activation (W. Herr, personal communication). Although Ser-Thr-rich regions are also present in Spl they are dispensible for transcriptional activation in *Drosophila* cells[24];

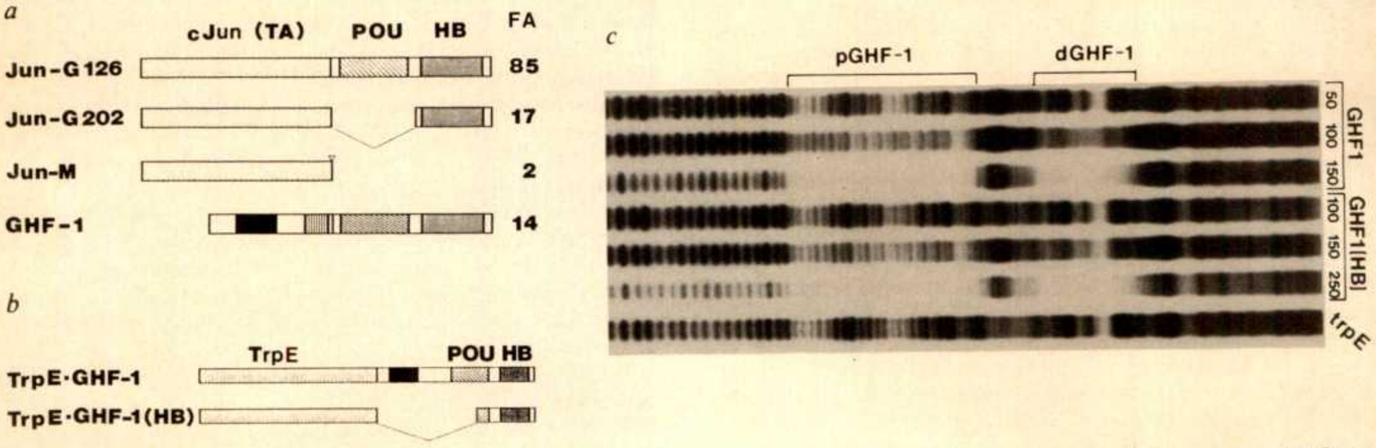
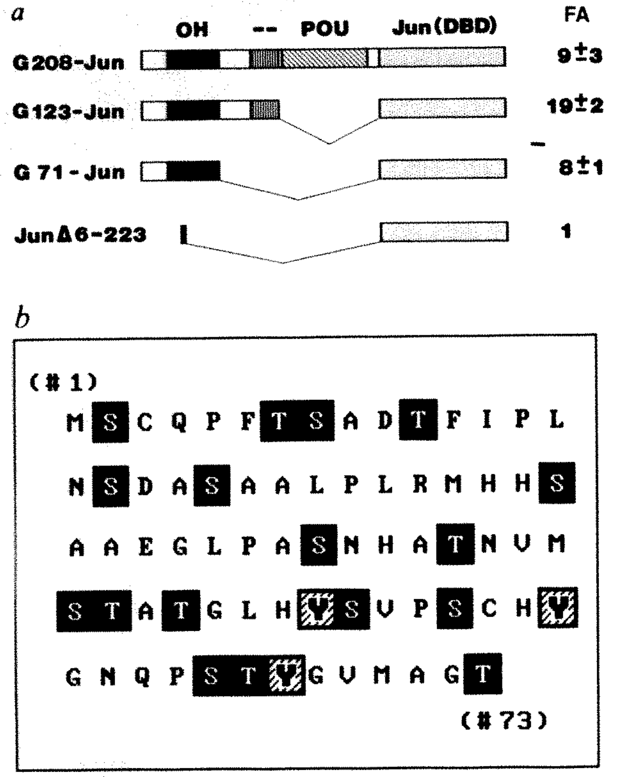


FIG. 3 Role of the POU-specific domain. *a*, Structure and activities of chimaeric Jun–GHF-1 constructs. Dotted box, c-Jun activation region (amino-acid residues 1–193); other symbols denote GHF-1 sequences as described in (Fig. 2). The activities of these constructs were determined by transfection of F9 cells with 10 μg of expression vector and 5 μg *GH-CAT* or *GHΔ(−82/−128)-CAT* lacking GHF-1-binding sites. Basal *GH-CAT* expression was determined by co-transfection with RSVcJunΔ6-223, which lacks the activation region[17] and was given an arbitrary value of 1. Fold activation (FA) was determined relative to this value and represents the average of two different experiments. Expression of *GHΔ(−82/−128)-CAT* was not affected by any of the expression vectors (data not shown). *b*, Structure of the TrpE-GHF-1 and TrpE-GHF(HB) fusion proteins. Dotted box, TrpE N-terminal region (M_r 37K); other boxes denote GHF-1 sequences as described above. *c*, Footprint analysis of the DNA-binding specificities of TrpE-GHF-1 and TrpE-GHF(HB). End-labelled human GH gene promoter fragment (+3 to −500; labelled at +3) was incubated with increasing amounts (ng) of the two fusion proteins as indicated, or 400 ng plasmid p 37^{trpE} and subjected to DNase I footprinting.

METHODS. To construct Jun-G126 and Jun-G202, *Bgl*II–*Asp*718 DNA fragments from *Δ72–125* and *Δ124–201* (containing the sequences encoding the GHF-1 POU–HB and HB, respectively) were inserted into *c-JunΔ194–223* (ref. 17) replacing the c-Jun DNA-binding domain. c-Jun-M contains the c-Jun activation domain (amino-acid residues 1–193) and lacks the DNA-binding domain[17]. Transfections were as described above. TrpE-GHF-1 was constructed by inserting a 0.9-kb *Bam*HI-*Hin*dIII fragment containing the complete GHF-1 gene coding region into the polylinker region of plasmid pATH3 which contains the TrpE N-terminal-encoding region, coding for p37^{trpE} (ref. 22). *trpE-GHF*(*HB*) contains an *Eco*RI fragment corresponding to GHF-1 amino-acid residues 164–291 as previously described[8]. Preparation of TrpE fusion proteins, gel-retardation assays (not shown), and DNase I footprinting reactions as previously described[8]. Amounts of proteins were determined by silver staining.


a
OH
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POU
Jun(DBD)
FA
G208-Jun
9±3
G123-Jun
19±2
G 71-Jun
8±1
JunΔ6-223
1
b
(#1)
M S C Q P F T S A D T F I P L
N S D A S A A L P L R M H H S
A A E G L P A S N H A T N V M
S T A T G L H Y S V P S C H Y
G N Q P S T Y G V M A G T
(#73)

FIG. 4 GHF-1 contains a novel activation domain. *a*, Structures and activities of chimaeric constructs, containing N-terminal fragments of GHF-1 fused to the DNA-binding domain of c-Jun (dotted box). The activities of these constructs were determined by transfections of F9 cells with 10 μg expression vector and 3 μg of *−73-Col-CAT*, which contains an AP-1-binding site[17,22] and assaying CAT activity 20 h later. Fold activation (FA) is relative to the basal expression of *−73-Col-CAT*, which was determined by co-transfection with *JunΔ6-223* (ref. 17) and represents the average of three separate experiments. *b*, Primary structure of the GHF-1 activation domain. The serine, threonine and tyrosine residues are highlighted (single-letter amino-acid code).
METHODS. To construct *G208-Jun*, *G123-Jun* and *G71-Jun*, a 1.6-kb *Bgl*II-*Asp*718 DNA fragment corresponding to the c-Jun DNA-binding domain was isolated from RSVcJunΔ194-223 (ref. 17) and inserted in-frame into *Δ209-252*, *Δ124-201* and *Δ72-125*, respectively.

but it is possible that in other cell types these regions are active. It is of interest that the STA domain has a similar composition to the C-terminal repeat of the largest subunit of RNA polymerase II (ref. 30). In addition to hydroxylated residues, these regions contain several prolines, indicating that they do not form α-helices. It is possible that the STA domain may contact directly, by hydrogen bonding, the C-terminal domain of the largest subunit of RNA polymerase II. Because the C-terminal domain of the largest subunit is subject to phosphorylation[31], it is more likely to make contact with the hydroxylated or basic amino-acid residues than acidic residues as previously suggested[32]. □

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1. Karin, M. in *Tissue Specific Gene Expression* (ed. Renkawitz, R.) 137–137 (VCH, Weinheim, 1989).
2. Miller, W. L. & Eberhardt, N. L. *Endocr. Rev.* **4**, 97–130 (1983).
3. Lefevre, C. *et al. EMBO J.* **6**, 971–981 (1987).
4. West, B. L. *et al. Molec. cell. Biol* **7**, 1193–1197 (1987).
5. Behringer, R. R., Mathews, L. S., Palmiter, R. D. & Brinster, R. L. *Genes Dev.* **2**, 453–461 (1988).
6. Borrelli, E. *et al. Nature* **339**, 538–541 (1989).
7. Bodner, M. & Karin, M. *Cell* **50**, 267–275 (1987).
8. Bodner, M. *et al. Cell* **55**, 505–518 (1988).
9. Dolle, P. *et al. Cell* (in the press).
10. Ingraham, H. A. *et al. Cell* **55**, 519–529 (1988).
11. Herr, W. *et al. Genes Dev.* **2**, 1513–1516 (1988).
12. Karin, M. *et al. Nature* **308**, 513–519 (1984).
13. Castrillo, J.-L., Bodner, M. & Karin, M. *Science* **243**, 814–817 (1989).
14. Desplan, C., Theis, J. & O'Farrell, P. H. *Cell* **54**, 1081–1090 (1988).
15. Hoey, T., & Levine, M. *Nature* **332**, 858–861 (1988).
16. Muller, M. *et al. EMBO J.* **7**, 4299–4304 (1988).
17. Angel, P., Smeal, T., Meek, J. & Karin, M. *New Biol.* **1**, 35–43 (1989).
18. Sturm, R. A. & Herr, W. *Nature* **336**, 601–604 (1988).
19. Ma, J. & Ptashne, M. *Cell* **51**, 113–119 (1987).
20. Hope, I. A., Mahadevan, S. & Struhl, K. *Nature* **333**, 635–640 (1988).
21. Ptashne, M. *Nature* **335**, 683–689 (1988).
22. Angel, P. *et al. Nature* **332**, 166–171 (1988).
23. Richardson, W. D., Roberts, B. L. & Smith, A. E. *Cell.* **44**, 77–85 (1986).
24. Courey, A. J. & Tjian, R. *Cell* **55**, 887–898 (1988).
25. Clerc, R. G. *et al. Genes Dev.* **2**, 1570–1581 (1988).
26. Muller, M. M., Rupert, S., Schaffner, W. & Matthias, P. *Nature* **336**, 544–551.
27. Scheidereit, C. *et al. Nature* **336**, 551–557 (1988).
28. Finney, M., Ruvzun, G. & Horvitz, H. R. *Cell* **55**, 757–769 (1988).
29. Braun, T. *et al. EMBO J.* **8**, 701–709 (1989).
30. Allison, L. A., Moyle, M., Shales, M. & Ingles, C. J. *Cell* **42**, 599–610 (1985).
31. Cisek, L. J. & Corden, J. L. *Nature* **339**, 679–684 (1989).
32. Sigler, P. S. *Nature* **333**, 210–212 (1988).
33. Mullis, K. S. & Faloona, F. A. *Meth. Enzym.* **155**, 335–350 (1987).
34. Hattori, M. & Sakaki, Y. *Analyt. Biochem.* **152**, 232–238 (1986).
35. Chen, C. & Okayama, H. *Molec. cell. Biol.* **7**, 2745–2752 (1987).
36. Maxwell, I. H., Harrison, G. S., Wood, W. M. & Maxwell, F. *Biotechnology* **7**, 276–280 (1980).

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A highly divergent HIV-2-related isolate

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IT has been suggested that the human immunodeficiency virus type 2 (HIV-2) and the simian immunodeficiency virus from rhesus macaques (SIV$_{\mathrm{mac}}$) evolved from the sooty mangabey virus SIV$_{\mathrm{sm}}$ (ref. 1). We now describe an HIV-2-related isolate, HIV-2$_{\mathrm{D205}}$, from a healthy Ghanaian woman that is genetically equidistant to the prototypic HIV-2 strains and to SIV$_{\mathrm{sm}}$ and SIV$_{\mathrm{mac}}$. Supported by the observation that HIV-2$_{\mathrm{D205}}$ differs in a step of envelope glycoprotein processing, our data indicate that it could represent an alternative HIV-2 subtype and that viruses of the HIV-2/SIV$_{\mathrm{sm}}$/SIV$_{\mathrm{mac}}$ group could have already infected humans before HIV-2 and SIV$_{\mathrm{sm}}$/SIV$_{\mathrm{mac}}$ diverged.

We have previously reported the molecular cloning of HIV-2$_{\mathrm{D205}}$ (ref. 2). This isolate from a healthy woman from Ghana was biologically characterized by its good growth on macrophages and by the absence of cytopathic effects on lymphocytes. It was originally classified as HIV-2 by western blot analysis.

We have now determined the complete nucleotide sequence of the clone HIV-2$_{\mathrm{D205,7}}$, which encompasses 7,817 base pairs (bp) of the proviral genome starting from the left long terminal repeat (LTR). We compared the sequence with the known HIV and SIV sequences (Table 1). Surprisingly, HIV-2$_{\mathrm{D205,7}}$ is only 76.2–76.8% identical to the other HIV-2 strains sequenced so far (strains ROD[3], D194[2], NIHZ[4] and ISY[5]). But the relationship of HIV-2$_{\mathrm{D205,7}}$ to SIV$_{\mathrm{sm}}$ (ref. 1; 76.4% nucleotide-sequence identity), SIV$_{\mathrm{mac}}$ (ref. 6; 75.0%), SIV$_{\mathrm{agm}}$ (ref. 7; 58.2%) and HIV-1$_{\mathrm{BRU}}$ (ref. 8; 57.8%) is in the same range as for the other HIV-2 isolates. HIV-2$_{\mathrm{D205}}$ is, therefore, genetically equidistant to the other HIV-2 strains and to SIV$_{\mathrm{sm}}$ and SIV$_{\mathrm{mac}}$. For comparison, the identity between the other HIV-2 strains is 87–90%, their identity to SIV$_{\mathrm{sm}}$ is 76.5–77.7%, and that to SIV$_{\mathrm{mac}}$ is 75.3–76.3%.

The genetic organization of HIV-2$_{\mathrm{D205}}$ is the same as for HIV-2, SIV$_{\mathrm{sm}}$ and SIV$_{\mathrm{mac}}$. Many of the open reading frames (*orf*) of HIV-2$_{\mathrm{D205}}$ differ in length only by one or a few codons from the *orf* of HIV-2$_{\mathrm{ROD}}$. The most notable difference is a

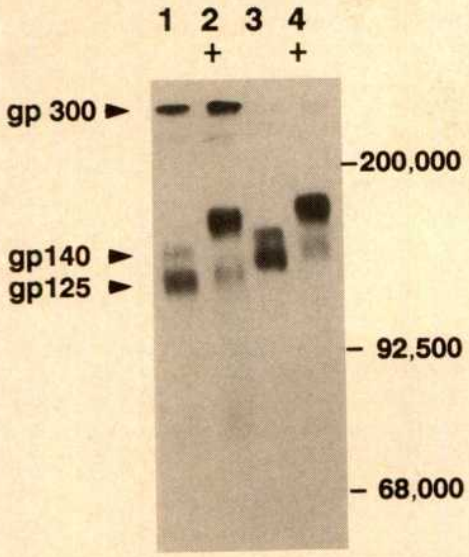

FIG. 1 Size comparison of the external glycoproteins (gp) of HIV-2_{ROD} and HIV-2_{D205}. [^{35}S]cysteine-labelled proteins from cells infected with HIV-2_{ROD} (lanes 1, 2) and HIV-2_{D205} (lanes 3, 4), immunoprecipitated by HIV-2-positive serum, and separated on an 8.5% SDS polyacrylamide gel. The external gp of HIV-2_{ROD} (gp125), its precursor (gp140), the dimeric form of gp140 (gp300), and the positions of M_r markers are indicated. In HIV-2_{D205}-infected cells, the band corresponding to the dimer is missing, even after treatment with castanospermine (lane 4), an inhibitor of glucosidase I, which causes the dimeric form to accumulate in HIV-2_{ROD} (lane 2). Notice that the external gp and its precursor are larger in HIV-2_{D205} than in HIV-2_{ROD}.

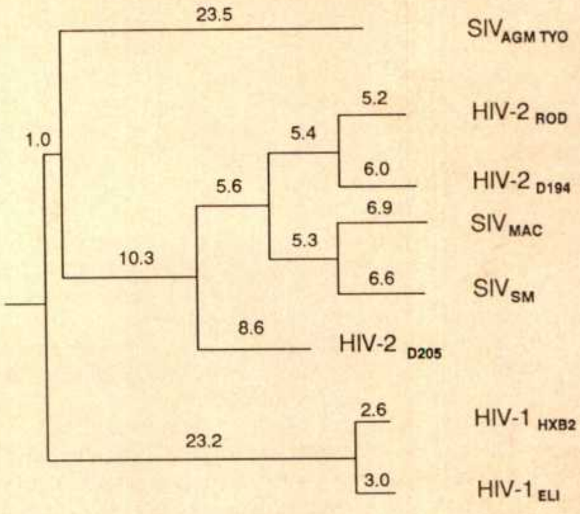

FIG. 2 Minimum-length evolutionary tree showing the relationship of HIV-2_{D205} to the HIV and SIV lentiviruses. The tree was generated from single base substitutions in the 3′ part of the HIV-$2_{D205.7}$ sequence using the PAUP program[13] and version 3.21 of the PHYLIP[14] bootstrapping algorithm. The tree was rooted on a random sequence. The total number of sites examined was 2,664; total number of variable sites, 1,548; consistency index, 0.746. Horizontal distances are proportional to the minimum number of single nucleotide substitutions required to generate the sequence examined or to the actual branch lengths indicated as fractional steps, that is, percentage of total sites.

54-bp insertion in the *gag-pol* overlap, followed by a 54-bp deletion, which puts the codons in phase again. This deletion, but not the insertion, is also present in the *gag-pol orf*s of SIV_{sm} and SIV_{mac}. In addition, the *gag-pol* overlap of HIV-2_{D205} is 60 nucleotides longer than it is in HIV-2_{ROD}, resulting in a *pol orf* that is extended by 20 codons at the 5′ end. We found some minor *orf*s by examining the DNA (+) strand. One of them, at position 1,851–1,651 on the lower strand of the HIV-$2_{D205.7}$ sequence, is conserved between all HIV-2 isolates, as well as in SIV_{mac} and SIV_{sm} (data not shown), and was preliminarily named *orf-y*. We predicted the deduced amino-acid sequence to constitute a small very hydrophobic protein of relative molecular mass 7,300 (M_r 7.3). Although there is, so far, no evidence for the transcription of the DNA (+) strand, of retroviruses, its remarkable conservation implies that it could have a function.

We compared the sequences of single genetic units from HIV-2_{D205}, HIV-2_{ROD}, SIV_{sm} and SIV_{mac}, and found that variability ranges from 4.6% (for genetic unit *R*) up to 35.9% (for *rev* exon 1) on the nucleotide level, and from 12.8% (for *gag*) up to 47.6% (for *rev* exon 1) at the amino-acid level (Table 2). But functionally relevant sequences or protein characteristics (such as hydrophilicity or charge) are highly conserved. For example, the identity of the complete LTR of HIV-2_{D205} to that of HIV-2_{ROD} is 84.5%, whereas the identity for TAR, the region necessary for the *trans*-activation by Tat[9,10], or the binding site for the transcription factor NF-κB (ref. 11), is 100%. The primer-binding site is 100% complementary to the 3′ end of the transfer RNA for lysine and the 'shifty sequence' TTTTTT, which catalyses the ribosomal frameshift between the *gag* and *pol orf*s, is unaltered. The sequenced part of the external glycoprotein of HIV-2_{D205} is 33% divergent from that of HIV-2_{ROD}, but has the same pattern of conserved and variable domains as the other HIV-2 and SIV_{sm}/SIV_{mac} strains[10]. All cysteine residues in the external glycoprotein of HIV-2_{ROD} are conserved. Divergence in the external glycoproteins is due to single amino-acid exchanges and small deletions or insertions, mainly in the variable regions. In addition, 8 out of 21 potential N-glycosylation signals differ between HIV-2_{D205} and HIV-2_{ROD}, indicating the importance of glycosylation in the generation of envelope variability.

To further characterize HIV-2_{D205}, we immunoprecipitated viral proteins and analysed them by SDS-PAGE. It is noteworthy that the envelope precursor glycoprotein, which forms a dimer in HIV-2 and SIV_{mac} to be processed correctly[12], was not dimeric in HIV-2_{D205} (Fig. 1). Furthermore, the external glycoproteins of HIV-2_{D205} and its precursor have higher apparent M_rs than those of HIV-2_{ROD}. This difference was also observed when glycosylation was inhibited (M.A., manuscript in preparation).

To determine the position of HIV-2_{D205} in the phylogenetic tree of lentiviruses, we constructed minimum-length evolutionary trees[13]. The tree based on the nucleotide variation in the 3′ part of the HIV-$2_{D205.7}$ sequence is shown in Fig. 2. HIV-2_{D205} is located remarkably close (8.6% difference) to a hypothetical

TABLE 1 Nucleotide sequence identity between HIV and SIV viruses (in %)

Strain	HIV-2 ROD	 D194	 NIHZ	 ISY	SIV sm	 mac	 AGM	HIV-1 BRU
HIV-$2_{D205.7}$	76.8	76.2	76.4	76.7	76.4	75.0	58.2	57.8
HIV-2_{ROD}	—	87.0	90.1	89.3	77.7	76.3	58.0	57.0
SIV_{mac251}	76.3	75.3	76.4	75.8	84.6	—	58.7	55.0

Sequences were obtained from the Los Alamos HIV Sequence Database[10] and compared using Microgenie sequence software from Beckman.

common ancestor of the HIV-2/SIV_{sm}/SIV_{mac} group of lentiviruses and branches before SIV_{sm}/SIV_{mac} diverged from the HIV-2 prototypes. In the tree, HIV-2_{D205} is 25% different from the prototypic HIV-2 viruses and 26% different from SIV_{sm} and SIV_{mac}, which agrees with our nucleotide-sequence comparison data in Table 1. This tree was congruent with the majority tree for the 5′ half of the genome. Bootstrapping, using version 3.21 of the PHYLIP algorithm[14], led to the same branching order in 40 out of 50 replicates. Branching length and branching order in this tree were in good agreement with those of other work (for example, see Fig. 4 of ref. 1). In the evolutionary tree analyses, the rarest occurrence was the clustering of HIV-2_{D205} with the prototypic HIV-2 viruses; sometimes HIV-2_{D205} clustered with SIV_{sm}/SIV_{mac}, but it usually appeared by itself as a close relative of the progenitor of both.

Our data indicate that the HIV-2/SIV_{sm}/SIV_{mac} group of viruses is older and genetically more divergent than previously believed. This antiquity, along with a high degree of variability, presumably accounts for the development of different subtypes: the HIV-2 prototypes, an alternative subtype defined by the strain HIV-2_{D205}, described here, and the simian strains SIV_{sm} and SIV_{mac}. The alternative subtype defined by HIV-2_{D205} is characterized by the high sequence divergence (24%) from that of the HIV-2 prototypes, and by the absence of dimer formation of the envelope protein precursor (Fig. 1), which is believed to be a necessary step in the processing of the envelope glycoproteins in the prototypic HIV-2 viruses and in SIV_{mac}. In this respect, HIV-2_{D205} behaves like HIV-1, in which no dimer formation has been observed.

To emphasize both the alternative subtype of HIV-2_{D205} and its evolutionary location close to a hypothetical common ancestor of the HIV-2/SIV_{sm}/SIV_{mac} group of lentiviruses, we propose that the virus is called HIV-2_{ALT}.

In our evolutionary tree, HIV-2_{ALT} is closely related to this common ancestor and branches earlier than SIV_{sm}/SIV_{mac} and the HIV-2 prototypes. Thus, the question of the origin of HIV-2 is open again. It is still unclear whether the host of a common ancestor of the HIV-2/SIV_{sm}/SIV_{mac} group was human or simian. A horizontal infection of a human by sooty mangabey seemed plausible because of the extent of sequence identity and the endemic coincidence[1,15]. But it must be noted that SIV_{sm} and SIV_{mac} are viruses taken from captive animals and that sequence data of SIV_{sm} from wild animals have not yet been published. Because captive monkeys were injected with human material back in the 1960's, artificial transmission from a human to a simian host could have occurred. It is possible, therefore, that SIV_{sm} and SIV_{mac} are fundamentally human viruses. In addition, the finding of HIV-2_{ALT}, a virus in a human, which is evolutionarily older than SIV_{sm}, could indicate that all subtypes of the HIV-2/SIV_{sm}/SIV_{mac} group are of human origin.

TABLE 2 Sequence conservation of genetic units (nucleotide/amino-acid)

HIV-2_{D205} genetic unit	% Identity to: HIV-2_{ROD}	SIV_{sm}	SIV_{mac251}
U3	72.9	66.8	63.5
R	95.4	94.3	91.6
U5	88.9	87.2	87.1
gag	81.5/84.5	82.5/87.2	80.6/83.7
pol	79.0/82.0	84.7/82.4	77.4/77.1
vif	72.4/68.9	72.9/68.7	72.0/61.0
vpx	76.1/75.2	76.4/75.2	75.2/77.9
vpr	78.8/69.8	74.6/73.4	78.9/76.4
tat exon 1	78.4/66.3	76.0/60.5	81.1/66.3
rev exon 1	76.1/56.5	64.1/52.4	70.0/60.9
env (partial)	70.0/67.0	68.8/65.6	68.6/65.7
nef (partial)	73.5/72.1	66.5/62.8	66.6/58.3

Identity between HIV-$2_{D205,7}$ and HIV-2_{ROD}, SIV_{sm} and SIV_{mac} allocated to single genes and LTR regions; *nef* from SIV_{sm} extends further downstream and was only compared up to the position corresponding to the stop codon of HIV-2 and SIV_{mac}.

On the basis of epidemiological data it has been proposed[16] that HIV-2, or at least a subtype of HIV-2, is less pathogenic in humans than is HIV-1. HIV-2_{ALT}, originating from a healthy Ghanaian, could be of this kind, as its position in the tree indicates that there exist viruses that had time to adapt to their human hosts. In addition, some individuals could have already evolved protective mechanisms that prevent certain HIVs from causing lethal disease. But out of the fifty cases of HIV-2 infection diagnosed in Germany so far, three have been fatal, one of them showing exclusively neurological symptoms[17]. Clearly, among viruses that serologically react like HIV-2, there are strains with very different genetic and biochemical properties. Thus, for further investigations on the spread of HIV-2 and its pathogenicity, it may be important to distinguish between the prototypic HIV-2 strains and the HIV-2_{ALT} subtypes. ☐

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1. Hirsch, V. M., Olmsted, R. A., Murphey-Corb, M., Purcell, R. H. & Johnson, P. R. *Nature* **339,** 389–392 (1989).
2. Kühnel, H. *et al. Proc. natn. Acad. Sci. U.S.A.* **86,** 2383–2387 (1989).
3. Guyader, M. *et al. Nature* **326,** 662–669 (1987).
4. Zagury, J. F. *et al. Proc. natn. Acad. Sci. U.S.A.* **85,** 5941–5945 (1988).
5. Franchini, G. *et al. Proc. natn. Acad. Sci. U.S.A.* **86,** 2433–2437 (1989).
6. Chakrabarti, L. *et al. Nature* **328,** 543–547 (1987).
7. Fukasawa, M. *et al. Nature* **333,** 457–461 (1988).
8. Wain-Hobson, S. *et al. Cell* **40,** 9–17 (1985).
9. Muesing, M., Smith, D. H. & Capon, D. J. *Cell* **48,** 691–701 (1987).
10. Myers, G. *et al. Human Retroviruses and AIDS* (Los Alamos National Lab., New Mexico, 1989).
11. Nabel, G. & Baltimore, D. *Nature* **326,** 711–713 (1987).
12. Rey, M. A. *et al. J. Virol.* **63,** 647–658 (1989).
13. Smith, T. F., Srinivasan, A., Schochetman, G., Marcus, M. & Myers, G. *Nature* **333,** 573–575 (1988).
14. Felsenstein, F. *A. Rev. Genet.* **22,** 521–566 (1988).
15. Doolittle, R. F. *Nature* **339,** 338–339 (1989).
16. Marlink, R. G. *et al. AIDS Res. hum. Retrovir.* **4,** 137–148 (1988).
17. Rübsamen-Waigmann, H. *et al. AIDS Forsch.* **2,** 572–575 (1987).

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Isolation and characterization of the G-actin–myosin head complex

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THE two main proteins involved in muscular contraction and cell motility, myosin and actin, possess the intrinsic property of being able to form filamentous structures[1]. This property poses a serious impediment to the study of their structures and interactions, and a considerable effort has thus been made to isolate their functional domains. The globular part of myosin, subfragment-1 (S1), which possesses ATPase and actin-binding sites as well as supporting the movement of actin filaments during *in vitro* assays, has been isolated[2–4]. But because S1 is efficient in inducing actin polymerization[5,6], as is myosin, it has not been possible to prepare and characterize a complex of S1 with monomeric actin (G-actin). We have now used chromatographically purified proteins to show that only the S1 isoenzyme carrying the A1 light-chain subunit promotes actin polymerization. The other isoenzyme, S1 (A2), carrying the A2 light-chain subunit, binds to actin, forming a tight complex of G-actin and S1 in a 1:1 ratio. This new functional difference

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between myosin isoforms directly implicates the A1 light-chain in myosin-induced actin polymerization. Additionally, this finding should lead to the purification of the stable G-actin–S1 complex needed to resolve the structure and to understand the molecular dynamics of the actin–myosin system.

Two principal myosin isoforms have been defined on the basis of the heterogeneity of the 'alkali' light-chain subunits, A1 and A2, bound to the globular heads of myosin[7]. Thus the two isoforms of the skeletal myosin head, S1(A1) and S1(A2), differ in the composition of their light chains: the A1 subunit of S1(A1) has an additional 41 N-terminal amino acids[8]. At low ionic strength, this difference alters both the actin-activated Mg^{2+}-dependent ATPase of S1, and the affinity of S1 for filamentous actin (F-actin)[9-11]. In more physiological conditions, and in the presence of the muscle regulatory system, this difference results mainly in a higher binding of S1(A1) isoenzyme to F-actin[12].

We first investigated whether both isoenzymes of S1 polymerize actin in the same fashion by measuring the amount of protein polymerized by each isoenzyme after a high-speed centrifugation (Fig. 1*A*). The addition of S1(A1) to a solution of G-actin induced the formation of filamentous actin co-sedimenting with an equimolar amount of S1(A1). When we used S1(A2), however, both actin and S1(A2) remained in the supernatant, indicating that actin polymerization had not occurred. By contrast, control experiments showed that both S1(A1) and S1(A2) normally bind to F-actin and cosediment with it (Fig. 1*A*, lanes d, Sd, e, Se). We established unequivocally the lack of actin polymerization in the presence of S1(A2) by performing light-scattering experiments (Fig. 1*B*). The addition of S1(A2) to G-actin did not modify the intensity of scattered light, confirming the absence of actin polymer in the solution. By contrast, S1(A1) gradually increased the intensity of light-scattering of the actin solution, demonstrating that actin polymerization had occurred. Further analysis of the last result showed that, in contrast to a previous report[13], the time course of the S1(A1)-induced polymerization resembles that of salt-induced polymerization[14]—that is, a lag phase, usually interpreted as the activation and nucleation phase of the actin monomer, precedes the elongation phase of the actin polymer.

Although S1(A2) does not polymerize actin, it does form a tight complex with monomeric actin. By measuring fluorescence polarization of chromophoric labels attached to actin, we could detect changes in the hydrodynamic volume of the protein, normally occurring during the formation of G-actin–S1 complexes or during actin polymerization[15]. When S1(A2) was added to G-actin labelled with 1,5-IAEDANS(5-[2-(iodoacetyl)-aminoethyl]aminonaphthalene-1-sulphonic acid), the polarization rapidly increased from its initial value of 0.23 to 0.33 (Fig. 2*a*). These values agree reasonably well with theoretical calculations, which give polarization values of 0.22 and 0.345 for the

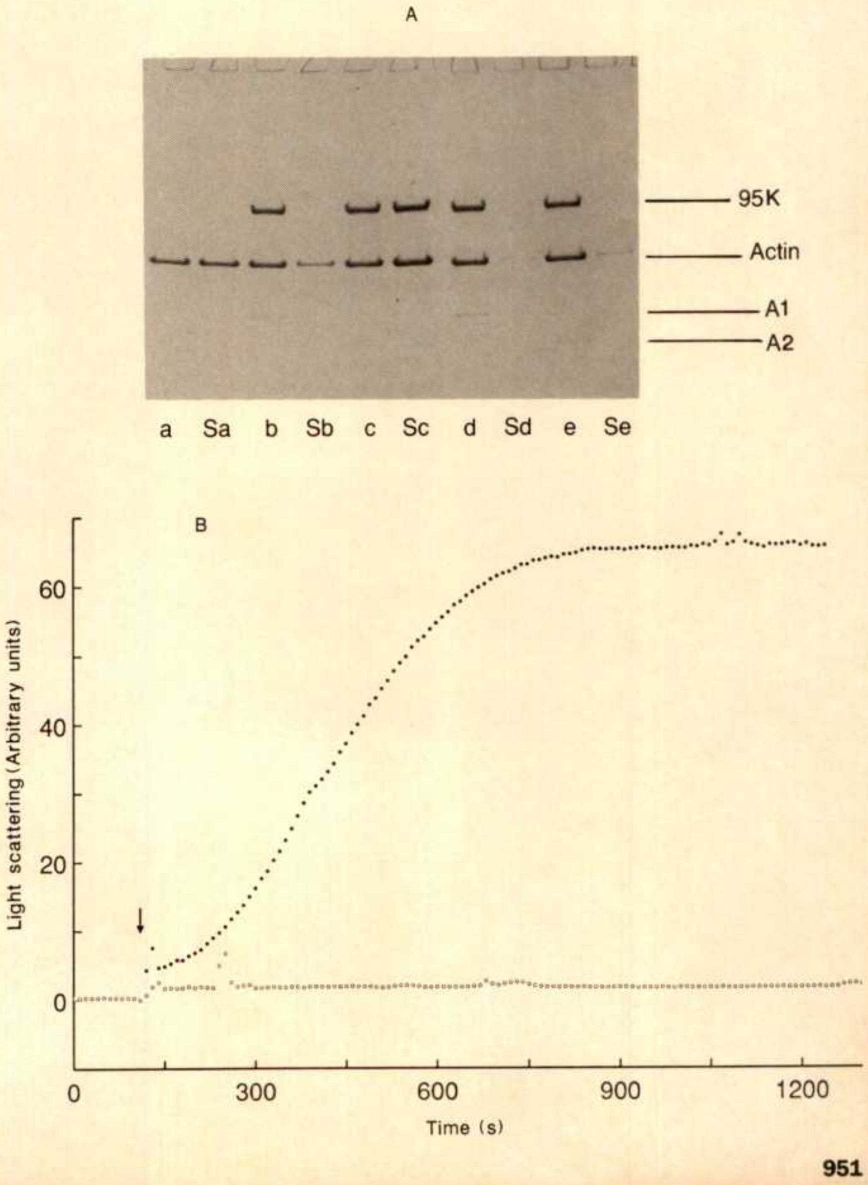

FIG. 1 A, G-actin polymerization followed by co-sedimentation experiments. a, G-actin alone (18 μM); b, d, G-actin mixed with S1(A1) (15 μM); or c, e, S1(A2) (15 μM); were centrifuged for 60 min at 160,000*g* after incubation for 30 min at 20 °C in G buffer (a, b, c) or in G buffer supplemented with 2 mM $MgCl_2$, 0.2 mM EGTA (d, e). Aliquots were withdrawn before (a–e) and after (Sa–Se) centrifugation and were loaded on 3.5–18% gradient polyacrylamide gel. Position of S1 heavy chain shown as 95K (*M*, 95,000). B, G-actin polymerization detected by light scattering. Time course of light scattering at 90° ($\lambda_{\text{excitation}}$ = 600 nm) of G-actin (11 μM) in G buffer observed after the addition (arrow), of 13 μM S1(A1) (●) or S1(A2) (○).

METHODS. S1 isoenzymes S1(A1) and S1(A2) were prepared from rabbit skeletal muscle myosin as previously reported[19] and were kept on ice in Tris-HCl buffer (30 mM), NaN_3 (0.1 mM), dithioerythritol (0.2 mM), pH 7.8. G-actin was extracted from the acetone powder of muscle of the same type as S1 (ref. 20), and further purified by G-150 gel chromatography using a column equilibrated with G buffer (Tris-HCl buffer, 2 mM; $CaCl_2$, 0.2 mM; ATP, 0.1 mM; DTE, 0.2 mM; NaN_3, 0.1 mM, pH 7.8). Protein concentrations were determined from absorbance[10,21].

monomeric actin and the G-actin–S1 complex, respectively[16]. For S1(A1), the polarization increased initially to 0.37, but rather than remaining stable, it further slowly increased to 0.44, the value characteristic for F-actin. We interpreted these observations as follows. When either of the S1 isoenzymes is mixed with G-actin, it forms a G-actin–S1 complex. Whereas the G-actin–S1(A2) complex does not change its aggregation state, the G-actin–S1(A1) complex has a strong tendency to form filamentous structures. This ability of the complex to polymerize must be due to the presence of the extra N-terminal residues of the A1 light chain, which were shown to directly interact with amino-acid residues 360, 362 and 364 of actin[17,18]. Whether actin polymerization results in the binding of the A1 light chain and the heavy chain to the same or to different actin monomers remains to be determined. Fluorescence polarization data, however, support the formation of a 1:1 G-actin–S1 complex in an early stage of the polymerization process. Furthermore, from fluorescence anisotropy titration of G-actin with S1(A2) (Fig. 2*b*), we determined that the number of S1(A2)-binding sites on G-actin per G-actin monomer is 1.1 and that the dissociation constant (K_d) for the complex formation is 0.4 μM.

Because of the sensitivity of the G-actin–S1(A2) complex to ATP, it is not possible to purify the complex in the ATP-containing buffer needed for G-actin stability. Therefore we developed a method of preparing a covalent G-actin–S1(A2) complex by crosslinking G-actin to S1(A2) at 4 °C using EDC (1-ethyl-3[3-(dimethylamino)-propyl]carbodiimide) as a catalyst. The purification of this complex by gel filtration chromatography yielded a product that was contaminated by traces of S1(A2) but entirely free of monomeric actin (Fig. 3). This complex was eluted from the column in about the same volume as was the relative molecular mass (M_r) standard (M_r 158,000), and therefore was certainly composed of G-actin and S1 in a 1:1 ratio (M_r 155,000), corroborating the results of the spectroscopic measurements.

Although a more complete investigation is needed to understand in detail the mechanism of the S1(A1)-induced actin polymerization, it is interesting to note that the A1 light chain is present only in myosin from skeletal or cardiac muscle. So, smooth muscle or nonmuscle myosins which possess A2-like light chains, may not polymerize actin. Whether this functional difference between the two S1 isoforms has physiological sig-

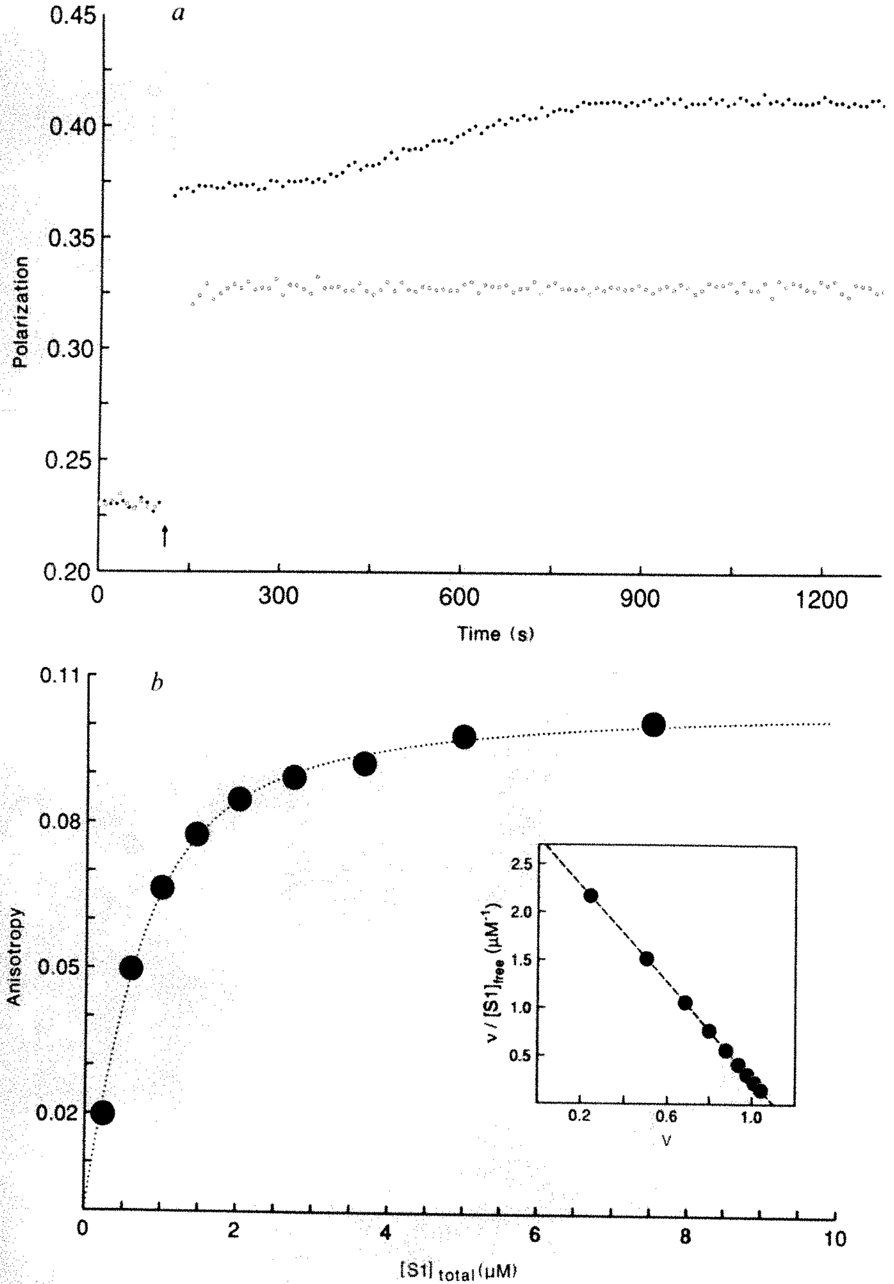

FIG. 2 *a*, Effect of S1(A1) and of S1(A2) on the fluorescence polarization of 1,5-IAEDANS-labelled actin. S1(A1) (13 μM; ●) or S1(A2) (13 μM; ○) were added (arrow) to a solution of 1,5-IAEDANS-labelled G-actin (11 μM) in G buffer. *b*, Titration curve of 0.5 μM 1,5-IAEDANS-labelled G-actin by S1(A2). The values of fluorescence anisotropy and the fraction of bound protein were calculated from polarization data as described[22]. Insert, Scatchard plot of the data where *v* is the fraction of S1 bound to actin.

METHODS. Actin was labelled with 1,5-IAEDANS according to ref. 23 and purified on a G-150 gel filtration column. Polarization measurements were performed at 20 °C on an SLM 8000 fluorometer.

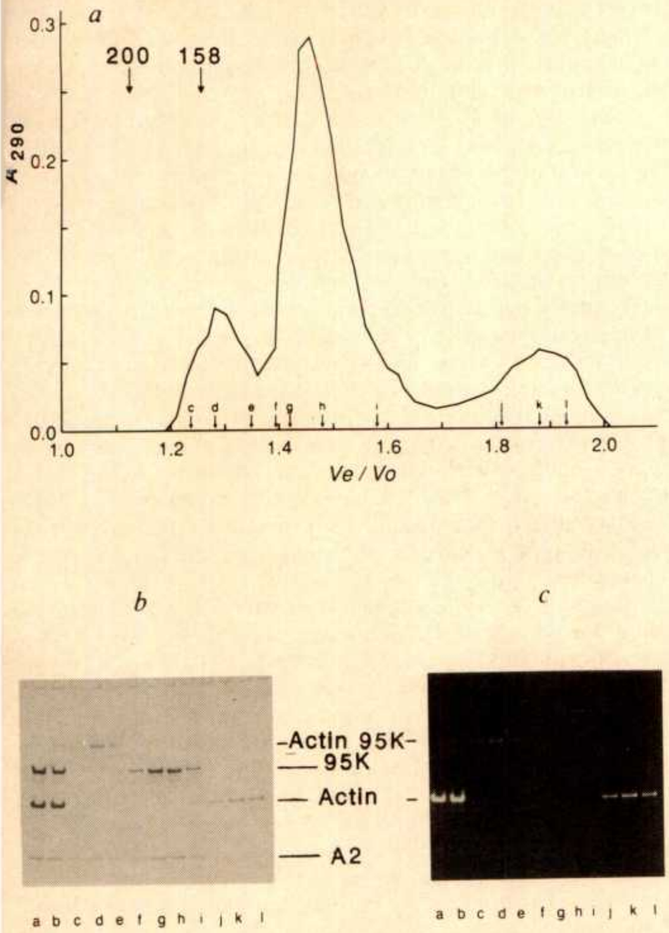

FIG. 3 Purification of the covalent G-actin–S1(A2) complex. *a*, Elution profile of EDC-crosslinked G-actin–S1(A2) complex from a Sephadex G-150 column. Abbreviations: V_e, elution volume; V_0, void volume. Aliquots of fractions c–l were loaded on SDS-PAGE and stained with Coomassie blue (*b*) or viewed in the ultraviolet light (*c*). Lanes a and b, composition of 1,5-IAEDANS-labelled G-actin–S1(A2) mixture before and after EDC-catalysed crosslinking reaction, respectively.

METHODS. 1,5-IAEDANS-labelled G-actin (40 μM) and S1(A2) (50 μM) were mixed in G buffer at 4 °C and a solution of EDC was added to a final concentration of 10 mM. During the 50 min of the crosslinking reaction, the mixture was centrifuged at 160,000*g*, 4 °C. The supernatant was then collected, the reaction was stopped with a 100-fold molar excess of DTE, and the sample was loaded on a G-150 column (2.5 × 140 cm) equilibrated with G buffer at 4 °C. M_r standards ($M_r \times 10^{-3}$): aldolase, 158; α-amylase, 200.

nificance is not clear, because in the presence of 0.5 mM $MgCl_2$, the G-actin-S1(A2) complexes spontaneously form filaments (data not shown). Nonetheless, both the noncovalent and covalent complexes described here are undoubtedly useful for further functional and structural studies, as they provide potential samples for the crystallization and the resolution of the three-dimensional structure of the native G-actin–myosin head complex. ☐

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1. Huxley, H. E. *J. molec. Biol.* **7,** 281–308 (1963).
2. Lowey, S. & Cohen, C. *J. molec. Biol.* **4,** 293–308 (1962).
3. Eisenberg, E. & Moos, C. *Biochemistry* **7,** 1486–1489 (1968).
4. Toyoshima, Y. Y. *et al. Nature* **328,** 536–539 (1987).
5. Yagi, K., Mose, R., Sakakibara, I. & Asai, H. *J. biol. Chem.* **240,** 2448–2454 (1965).
6. Cooke, R. & Morales, M. F. *J. molec. Biol.* **60,** 249–261 (1971).
7. Weeds, A. G. & Lowey, S. *J. molec. Biol.* **61,** 701–725 (1971).
8. Frank, G. & Weeds, A. G. *Eur. J. Biochem.* **44,** 317–332 (1974).
9. Weeds, A. G. & Taylor, R. S. *Nature* **257,** 54–56 (1975).
10. Wagner, P. D. & Weeds, A. G. *J. molec. Biol.* **109,** 455–473 (1977).
11. Chalovich, J. M., Stein, L. A., Greene, L. E. & Eisenberg, E. *Biochemistry* **23,** 4885–4889 (1984).
12. Trayer, H. R. & Trayer, I. P. *FEBS Lett.* **180,** 170–174 (1985).
13. Miller, L., Phillips, M. & Reisler, E. *J. biol. Chem.* **263,** 1996–2002 (1988).
14. Oosawa, F. *Muscle and Nonmuscle Motility,* 151–216 (Academic, New York, 1983).
15. Perrin, F. *Ann. Phys.* **12,** 169–173 (1929).
16. Chaussepied, P. & Kasprzak, A. A. *J. biol. Chem.* (in the press).
17. Sutoh, K. *Biochemistry* **21,** 3654–3661 (1982).
18. Henry, G. D. *et al. Biochim. biophys. Acta* **830,** 233–243 (1985).
19. Chaussepied, P. & Morales, M. F. *Proc. natn. Acad. Sci. U.S.A.* **85,** 7471–7475 (1988).
20. Spudich, J. A. & Watts, S. *J. biol. Chem.* **246,** 4866–4871 (1971).
21. Houk, T. W. & Hue, K. *Analyt. Biochem.* **57,** 453–459 (1974).
22. Kasprzak, A. A. & Villafranca, J. J. *Biochemistry* **27,** 8050–8056 (1988).
23. Kasprzak, A. A., Takashi, R. & Morales, M. F. *Biochemistry* **27,** 4512–4522 (1988).

Sensitivity to cyclosporin A is mediated by cyclophilin in *Neurospora crassa* and *Saccharomyces cerevisiae*

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CYCLOSPORIN A, a cyclic fungal undecapeptide produced by *Tolypocladium inflatum*, is a potent immunosuppressive drug originally isolated as an antifungal antibiotic[1,2]. Cyclosporin A (CsA) is widely used in humans to prevent rejection of transplanted organs such as kidney, heart, bone marrow and liver[3]. The biochemical basis of CsA action is not known: its primary cellular target has been suggested to be calmodulin[4,5], the prolactin receptor[6,7] or cyclophilin, a CsA-binding protein originally isolated from the cytosol of bovine thymocytes[8–10]. Cyclophilin has been shown to be a highly conserved protein present in all eukaryotic cells tested[11–15] and to be identical[16,17] to peptidyl–prolyl *cis-trans* isomerase[18,19], a novel type of enzyme[20] that accelerates the slow refolding phase of certain proteins *in vitro*[21,22]. We demonstrate that in the lower eukaryotes *N. crassa* and *S. cerevisiae*, cyclophilin mediates the cytotoxic CsA effect. In CsA-resistant mutants of both organisms, the cyclophilin protein is either lost completely or, if present, has lost its ability to bind CsA.

We selected for CsA-resistant mutants in the St Lawrence 74A wild-type strain of *N. crassa* by applying the lowest CsA concentration (1 μg ml^{-1}) found to prevent growth. Forty-one ultraviolet-induced mutant strains were isolated and four of those, belonging to a high-resistance class (B9; B12; B27; B32; all growing on 10 μg ml^{-1} CsA), were crossed to a double-mutant strain *pan-2 arg-12* to combine the CsA-resistance with 'forcing markers' for heterokaryon formation. We performed the same procedure with a spontaneous mutant (B60) which grew on 5 μg ml^{-1} CsA. In all five crosses, CsA-resistant and CsA-sensitive segregants occurred in a 1:1 ratio, indicating the involvement of a single gene in each case. Segregants that carried each mutation in wild-type background were selected for biochemical analysis (Fig. 1); segregants with mutations in combination with either *pan-2* or *arg-12* markers and mating type *A* were selected for dominance and complementation tests. We found all five CsA-resistant mutations to be recessive in mycelia that were heterokaryotic for either one of them and the CsA-sensitive wild-type allele. Mycelia heterokaryotic for pairs of CsA-resistant mutations displayed the CsA-resistant phenotype, that is, they were unable to complement each other. Apparently the five mutations are allelic, indicating that a single gene, which we name *csr-1*, is responsible for CsA-resistance. Because we noticed a weak linkage (~33%) between CsA-resistant mutations and mating type *a*/*A* alleles (located on *N. crassa* linkage group I), mutant B12 was crossed to markers on chromosome I, that is *nit-2* (left arm) and *his-2* (right arm). We observed linkage with *his-2* with 19.5% recombination. The linkage with the mating type locus, and *his-2* allows us to position *csr-1* distal from *his-2* on the right arm of chromosome I.

All mutants grew normally at 25 °C. We also tested them for growth at 29 °C and 37 °C, but no temperature-sensitivity was observed.

In wild-type strains of *N. crassa* cyclophilin is located in both cytosol and mitochondria[14]. Both forms are encoded by a single nuclear gene, which is transcribed into two messenger RNAs with different 5'-ends. The shorter mRNA codes for the cytosolic form of relative molecular mass 20,000 (M_r 20K), whereas the longer mRNA codes for a 24K precursor protein whose N-terminal presequence is cleaved, in two steps, on import into mitochondria, leading to the mature 20K cyclophilin protein in the matrix[14].

To analyse whether the mutations leading to CsA-resistance affected the levels of cylophilin, we performed western blot assays[23] with cyclophilin-specific antibodies[14], as well as CsA-binding assays[8,9,11] (Fig. 1). Both cytosolic and mitochondrial fractions of wild-type strain and the CsA-resistant segregants in wild-type background (to ensure that only the *csr-1* gene was affected), were analysed. Cyclophilin was present in both the cytosolic and mitochondrial fractions of the wild-type strain 74A and the spontaneous mutant B60. None of the other mutants, however, contained immunodetectable cyclophilin in either the cytosol or the mitochondria (Fig. 1*a*).

In all the mutants we tested (including B60), no significant CsA-binding activity was detectable in the cytosolic fractions (Fig. 1*b*), in contrast to wild-type cytosol where normal binding was observed[14]. In mitochondrial extracts of all mutant strains (prepared by lysing isolated mitochondria in 1.8% *N*-octylglucoside and centrifugation[14]), specific CsA-binding activity was reduced to 10–30% of that seen with wild-type mitochondrial extracts[14]. The residual binding activity present in the mutant mitochondria may be due to another CsA-binding component, different from cyclophilin.

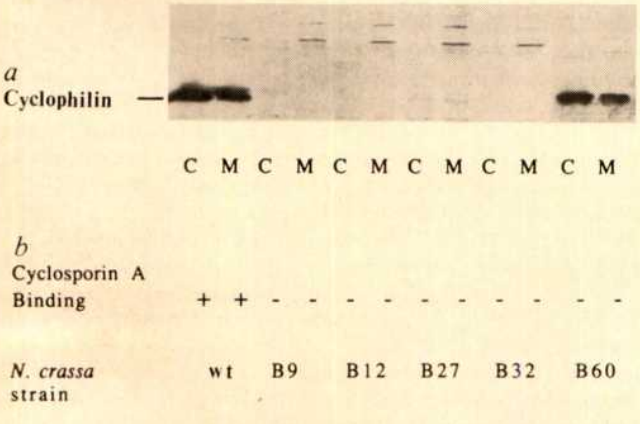

FIG. 1 Western blot analysis of cyclophilin (20K) (*a*) and analysis of CsA-binding (*b*) of cytosolic and mitochondrial fractions of *N. crassa* wild-type and CsA-resistant mutants.

METHODS. *a*, *N. crassa* 74A wild-type (wt) and CsA-resistant mutants (B9, B12, B27, B32, B60) were fractionated into cytosol (C) and mitochondria (M) (refs 14, 26). Protein (100 μg) from each fraction was electrophoresed on a 15% SDS-polyacrylamide gel, transferred to nitrocellulose paper[23], and immunodecorated with polyclonal antibodies raised against *N. crassa* cyclophilin[14]. Bound antibodies were visualized using peroxidase coupled to antibodies against rabbit IgG. *b*, Cytosolic fractions of the different strains were assayed for CsA binding[8,9,11,14]. With all mutants tested, specific binding of $[^3\mathrm{H}]$CsA was less than 5–10% of that of wild-type cytosol[14]. Isolated mitochondria were lysed in 1.8% *N*-octylglucoside, centrifuged[14] and the detergent extracts tested for the ability to bind $[^3\mathrm{H}]$CsA in a LH-20 assay[8,9,11,14]. In the mitochondrial extracts of the different mutants, specific binding of $[^3\mathrm{H}]$CsA was significantly reduced (10–30% of specific binding of wild-type mitochondrial extracts[14]).

Taken together, CsA-resistant mutants of *N. crassa* that are affected in the gene *csr-1* (which is probably the gene coding for cyclophilin) had either completely lost cyclophilin in both cytosol and mitochondria, or, if the protein was still present (as in mutant B60), it had lost its CsA-binding ability. These data indicate that either cytosolic or mitochondrial cyclophilin, or both, is the mediator of CsA action in *N. crassa*.

We performed similar experiments in the yeast *S. cerevisiae*, which was previously reported to be insensitive to CsA (ref. 1). We found that CsA-sensitivity seems to be coupled to a Petite phenotype, and is most probably due to alterations in the cell wall[24] which lead to increased permeability to the hydrophobic drug. Forty-eight different laboratory strains of *S. cerevisiae* were tested for CsA-sensitivity; only two, both with the Petite phenotype, were found to be sensitive to $100\,\mu g\,ml^{-1}$ CsA. CsA-sensitive mutants could easily be selected from CsA-resistant wild-type strains by screening for spontaneous Petite phenotypes.

Starting with the CsA-sensitive ρ^0 strain IL 993/5c (ref. 25) (wild type (wt); Fig. 2), eight independent, spontaneous CsA-resistant mutants were isolated (strains 1–8; Fig. 2). Growth tests on nonfermentable carbon sources such as glycerol, confirmed that they did not represent ρ^+ revertants. All CsA-resistant yeast mutants showed no significant CsA-binding activity in the cytosolic fractions (Fig. 2*b*). In addition, two of these mutants (mutants 7 and 8; Fig. 2*a*) contained no immunodetectable cyclophilin. Yeast cyclophilin has an apparent relative molecular mass of 17K, and is recognized by antibodies raised against either *N. crassa* cyclophilin[14] or isolated yeast cyclophilin. We have cloned and sequenced both a full-length complementary DNA and the gene coding for the cytosolic form of *S. cerevisiae* cyclophilin (K. Dietmeier and M. T., manuscript in preparation).

Our results demonstrate that cyclophilin is a cellular target of CsA. Both in *N. crassa* and in yeast the cytotoxic action of CsA is mediated by cyclophilin. In the absence of cyclophilin (or in the presence of a mutated cyclophilin that has lost its CsA-binding activity), CsA does not have any toxic effect.

These findings raise several intriguing questions concerning both the physiological role of cyclophilin and the mechanism

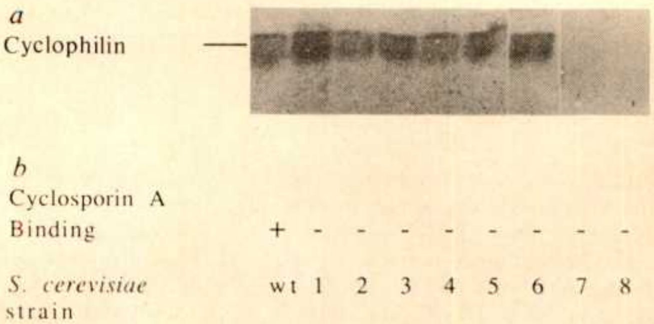

FIG. 2 Western blot analysis of cyclophilin (17K) (*a*) and analysis of CsA-binding (*b*) of cytosolic fractions of *S. cerevisiae* wild-type and spontaneous CsA-resistant mutants.

METHODS. *a*, Wild-type (wt) strain IL 993/5c (ref. 24) was grown at 30 °C in YPD medium[32], 1% ethanol, 0.1% Tween 80; eight independent spontaneous mutants (strains 1–8) were grown under identical conditions, except that the medium contained $50\,\mu g\,ml^{-1}$ CsA. Zymolyase-spheroplasts were lysed and cytosolic fractions prepared[32] and analysed as in Fig. 1. *b*, Cytosolic fractions of the different strains were assayed for CsA-binding[8,9,11,14]. With all mutants tested (strains 1–8), specific binding of $[^3\mathrm{H}]$CsA was less than 5–10% of that of wild-type cytosol[14].

of CsA action. Cyclophilin is a highly conserved protein in all eukaryotes analysed so far[9,12-14]. How can *N. crassa* and yeast cells survive without showing any detectable phenotype in the absence of cyclophilin?

Recent work[16,17] has demonstrated the identity of cyclophilin to peptidyl-prolyl *cis-trans* isomerase (PPIase)[18,19]. Both isolated *N. crassa* and yeast wild-type cyclophilins were found to possess PPIase-activity similar to the human and bovine enzymes (S. Mayer, F. X. Schmid, D. W. Nicholson and M.T., manuscript in preparation). Furthermore, PPIase-activity measured in cytosolic and mitochondrial fractions of *N. crassa* mutants that lack cyclophilin, indicate the presence of additional PPIase-activities in both the cytosol and mitochondria. In a Southern blot analysis of *N. crassa* DNA, using low-stringency conditions, we have found two bands that cross-hydridize with a cyclophilin probe, and which may correspond to the additional PPIase-activities. The presence of these additional PPIase-activities could explain why a complete loss of cyclophilin does not lead to cell death.

Why does CsA stop growth of wild-type cells but not of cyclophilin-deficient mutant cells? We propose that binding of CsA to cyclophilin leads to the formation of a complex which then interacts with another, unidentified cellular component. This interaction must be lethal to the cell. Other explanations are possible, although less likely. For example, cyclosporin A could be modified by cyclophilin and the derivative could be lethal to the cells.

It is tempting to speculate that cyclophilin may be involved in the unfolding/refolding of newly synthesized proteins, in particular of those which have to traverse the various cellular membranes[26-29]. In this respect it may be relevant that a cyclophilin-related protein, namely the product of the *ninaA* gene of *Drosophila melanogaster*, has been suggested to be involved in the folding of rhodopsin *in vivo*[30,31].

Note added in proof: We have learned that T. Chang, B. Weisblum and R. L. Metzenberg (personal communication) have also identified a *N. crassa* gene for cyclosporin A resistance on linkage group I, left of *cyh-1* (1%). □

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1. Dreyfuss, M. *et al. Eur. J. appl. Microbiol.* **3,** 125–133 (1976).
2. Borel, J. F., Feurer, C., Gubler, H. U. & Stähelin, H. *Agents and Actions* **6,** 468–475 (1976).
3. Beveridge, T. in *Prog. Allergy* **38** (ed. Borel, J. F.) 269–292 (Karger, Basel, 1986).
4. Colombani, P. M., Robb, A. & Hess, A. D. *Science* **228,** 337–339 (1985).
5. Colombani, P. M. & Hess, A. D. *Science* **233,** 988–989 (1986).
6. Larson, D. F. in *Prog. Allergy* **38,** (ed. Borel, J. F.) 222–238 (Karger, Basel, 1986).
7. Russel, D. H. *et al. Biochem. biophys. Res. Commun.* **121,** 899 (1984).
8. Handschumacher, R. E., Harding, M. W., Rice, J., Drugge, R. J. & Speicher, D. W. *Science* **226,** 544–547 (1984).
9. Harding, M. W., Handschumacher, R. E. & Speicher, D. W. *J. biol. Chem.* **261,** 8547–8555 (1986).
10. Hait, W. N., Harding, M. W. & Handschumacher, R. E. *Science* **233,** 987–988 (1986).
11. Koletsky, A. J., Harding, M. W. & Handschumacher, R. E. *J. Immun.* **137,** 1054–1059 (1986).
12. Haendler, B., Hofer-Warbinek, R. & Hofer, E. *EMBO J.* **6,** 947–950 (1987).
13. Danielson, P. E. *et al. DNA* **7,** 261–267 (1988).
14. Tropschug, M. *et al. J. biol. Chem.* **263,** 14433–14440 (1988).
15. Foxwell, B. M. J. *et al. Biochim. biophys. Acta* **938,** 447–455 (1988).
16. Takahashi, N., Hayano, T. & Suzuki, M. *Nature* **337,** 473–475 (1989).
17. Fischer, G., Wittmann-Liebold, B., Lang, K., Kiefhaber, T. & Schmid, F. X. *Nature* **337,** 476–478 (1989).
18. Fischer, G., Bang, H. & Mech, C. *Biomed. biochim. Acta* **43,** 1101–1111 (1984).
19. Fischer, G. & Bang, H. *Biochim. biophys. Acta* **828,** 39–42 (1984).
20. Freedman, R. B. *Nature* **337,** 407–408 (1989).
21. Lang, K., Schmid, F. X. & Fischer, G. *Nature* **329,** 268–270 (1987).
22. Lang, K. & Schmid, F. X. *Nature* **331,** 453–455 (1987).
23. Burnette, W. N. *Analyt. Biochem.* **112,** 195–203 (1981).
24. Evans, I. H., Diala, E. S., Earl, A. & Wilkie, D. *Biochim. biophys. Acta* **602,** 201–206 (1980).
25. Wolf, K., Dujon, B. & Slonimski, P. P. *Molec. gen. Genet.* **125,** 53–90 (1973).
26. Pfanner, N., Tropschug, M. & Neupert, W. *Cell* **49,** 815–823 (1987).
27. Eilers, M. & Schatz, G. *Cell* **52,** 481–483 (1988).
28. Tropschug, M. & Neupert, W. in *Anion Carriers of Mitochondrial Membranes* (eds Azzi, A., Nalecz, K. A., Nalecz, M. J. & Wojtczak, L.) 295–306 (Springer, Berlin, 1989).
29. Zimmermann, R. & Meyer, D. I. *Trends biochem. Sci.* **11,** 512–515 (1986).
30. Shieh, B.-H., Stamnes, M. A., Seavello, S., Harris, G. L. & Zuker, C. S. *Nature* **338,** 67–70 (1989).
31. Schneuwly, S. *et al. Proc. natn. Acad. Sci. U.S.A.* **86,** 5390–5394 (1989).
32. Cheng, M. Y. *et al. Nature* **337,** 620–625 (1989).

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Style self-incompatibility gene products of *Nicotiana alata* are ribonucleases

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SELF-INCOMPATIBILITY in flowering plants is often controlled by a single nuclear gene (the *S*-gene) having several alleles[1]. This gene prevents fertilization by self-pollen or by pollen bearing either of the two *S*-alleles expressed in the style. Sequence analysis shows that three alleles of the *S* gene of *Nicotiana alata* encode style glycoproteins[2,3] with regions of defined homology. Two of the homologous regions also show precise homology with ribonucleases T_2 (ref. 4) and Rh (ref. 5). We report here that glycoproteins corresponding to the S_1, S_2, S_3, S_6 and S_7 alleles isolated from style extracts of *N. alata*[6] are ribonucleases. These style *S*-gene-encoded glycoproteins account for most of the ribonuclease activity recovered from style extracts. The ribonuclease specific activity of style extracts of the self-incompatible species *N. alata* is 100–1,000-fold higher than that of the related self-compatible species *N. tabacum*. These observations implicate ribonuclease activity in the mechanism of gametophytic self-incompatibility.

Cloned complementary DNAs corresponding to the S_2-, S_3- and S_6-allelic glycoproteins[6] have recently been isolated[2,3]. The primary amino-acid sequences of three *S*-glycoproteins show some regions that are perfectly conserved and other regions that are less homologous[3]. Sequence analysis reveals significant homology between the *S*-glycoproteins and the extracellular ribonucleases (RNases) T_2 of *Aspergillus oryzae*[4] and Rh of *Rhizopus niveus*[5] (Fig. 1). The two longest regions of homology, residues 67–72 and 138–142 (Fig. 1) include His 67 and His 139 which are implicated in RNase T_2 catalysis[4]. It is significant that these homologous regions are also conserved among the three *S*-glycoproteins. In total, of the 122 amino acids perfectly conserved among the three *S*-glycoproteins, 30 are aligned with identical amino acids in the fungal RNases and another 22 are aligned with closely related amino acids. The positions of 5 out of 10 cysteine residues are conserved between the *S*-glycoproteins and the RNases. Two of these cysteine residues, 86 and 143, located in the vicinity of the proposed RNase active site, form a disulphide bond in RNase T_2 (ref. 4). These sequence similarities indicate a close structural relationship between the active-site domain of the two secreted fungal RNases and the homologous region of the *S*-glycoproteins. This homology prompted us to investigate the RNase activity of the *S*-glycoproteins.

The *S*-glycoproteins can be purified from style extracts by a procedure involving ammonium-sulphate fractionation and cation-exchange chromatography[6]. The *S*-glycoproteins isolated by this method behave as a single species on SDS-PAGE or reverse-phase HPLC; N-terminal sequencing confirms their identity with the proteins predicted from the cloned cDNAs[6]. The association of particular *S*-glycoproteins with the corresponding *S* allele is further supported by data which show that, in every case examined, the elution profile of proteins resolved by cation-exchange chromatography correctly predicts the genotype of the source material. Also, these isolated *S*-glycoproteins are active as inhibitors of *in vitro* pollen tube growth[7]. Initially, three *S*-glycoprotein preparations isolated from style extracts of plants homozygous for the S_1, S_2 and S_6 alleles had RNase activities when assayed by the perchloric acid precipitation method[8] (data not shown). The RNase activity of the isolated S_2-glycoprotein had a pH optimum of 7.0, a temperature optimum of 50 °C, and was not affected by Ca^{2+}, Mn^{2+}, Zn^{2+}, EDTA (1 mM) or Mg^{2+} (10 mM). Denatured DNA was not hydrolysed by the S_2-glycoprotein.

In two experiments (Fig. 2*a*, *b*), extracts from equal numbers of styles homozygous for two different alleles (S_3 plus S_7; S_1 plus S_6) were mixed and analysed. In all cases, RNase activity coeluted with the individual *S*-glycoproteins and was *S*-allele specific (Fig. 2, compare *b* and *c*). Given that the different *S*-glycoproteins are clearly resolved by ion-exchange chromatography and that the RNase activity coelutes with the *S*-glycoproteins, the ribonuclease activity must be an inherent component of the *S*-glycoproteins. Furthermore, the RNase specific activity of the purified *S*-glycoproteins varies from 170 (S_1-glycoprotein) to 2,200 (S_7-glycoprotein) A_{260} units min^{-1} mg^{-1}, values similar to the specific activity of purified RNase T_2 (3,900 A_{260} units min^{-1} mg^{-1}) when measured under the same conditions. The origin and possible biological significance of the variation in *S*-glycoprotein RNase specific activity, which accounts for the difference in the sizes of the RNase activity peaks (compare the S_6- and S_1-glycoprotein peaks in Fig. 2*b*) associated with the various *S*-glycoproteins, is now being investigated. This variation could be due to the differential stability of the RNase activities under purification conditions, which were designed primarily to optimize separation of the *S* allele-specific glycoproteins.

Because most of the RNase activity in crude style extracts was applied to the cation-exchange column (Fig. 2 legend) the percentage of the RNase activity recovered from the column was a good indicator of the proportion of the total style activity that is attributable to the *S*-glycoproteins. Of the recovered RNase activity, 40% (Fig. 2*c*) to 86% (Fig. 2*b*) can be attributed to *S*-glycoproteins. *N. alata* style extracts also contain an additional minor RNase activity (X in Fig. 2) which does not cofractionate with the *S*-glycoproteins on chromatography and is therefore not an allele-specific RNase.

TABLE 1 Ribonuclease specific activities

	Style extract*	Pollen extract*
N. alata		
S_1S_1	180	0.7
S_2S_2	240	—
S_3S_3	400	—
S_6S_6	300	0.7
S_7S_7	1,400	—
N. tabacum		
cv. Wisconsin 38	1.4	

Style extracts were prepared as described in Fig. 2 legend, but were not ammonium-sulphate fractionated. Pollen extracts were prepared similarly, except that pollen was disrupted by brief sonication. Ribonuclease activity was assayed at 37 °C according to Brown and Ho[8]. Protein was determined according to Bradford[10] using BSA as a standard. Abbreviation: cv, cultivar.

* A_{260} units released per min per mg.

This inherent association of RNase activity with the *S*-glycoproteins indicates a way in which they could function. The *S*-glycoproteins are secreted into the style mucilage, which separates the files of transmitting tissue cells and provides a matrix through which pollen tubes grow towards the ovary[3,9]. The style *S*-glycoprotein could penetrate the pollen tube and degrade RNA in the cytoplasm. This would interfere with protein synthesis, and could account for the characteristic slowing of self-pollen tube growth.

The nature of gametophytic self-incompatibility indicates that the *S*-gene must be expressed in the pollen as well as in the style[1]. Relative to style extracts, only trace amounts of RNase activity were detected in pollen extracts (Table 1) and in pollen germinated *in vitro* (B.A.M., unpublished results) indicating that, if it is an RNase, the product of the *S* gene in pollen is not highly expressed. Indeed, it now seems very unlikely that the *S*-gene products in the *N. alata* pollen and style are identical, yet it is clear that they must share an allelic specificity for recognition to occur[1]. It is possible that the specificity controls the access of style RNase to the putative substrate within the pollen tube. Alternatively, the specificity could reside in the

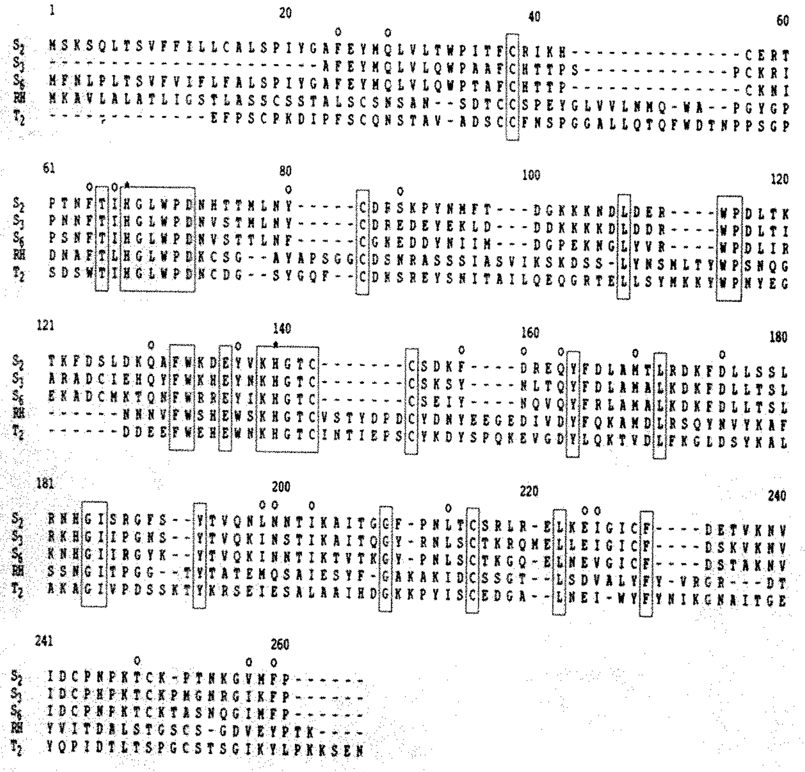
1
20
40
60
S2 MSKSQLTSVFFILLCALSPIYGAFEYMQLVLTWPITFCRIKH--------------CERT
S3 ----------------------AFEYMQLVLQWPAAFCHTTPS-------------PCKRI
S6 MFNLPLTSVFVIFLFALSPIYGAFEYMQLVLQWPTAFCHTTP--------------CKNI
RH MKAVLALATLIGSTLASSCSSTALSCSNSAN--SDTCCSPEYGLVVLNMQ-WA--PGYGP
T2 ---------------EFPSCPKDIPFSCQNSTAV-ADSCCFNSPGGALLQTQFWDTNPPSGP
61
80
100
120
S2 PTNFTIHGLWPDNHTTMLNY-----CDFSKPYNMFT---DGKKKNDLDER----WPDLTK
S3 PNNFTIHGLWPDNVSTMLNY-----CDREDEYEKLD---DDKKKKDLDDR----WPDLTI
S6 PSNFTIHGLWPDNVSTTLNF-----CGNEDDYNIIM---DGPEKNGLYVR----WPDLIR
RH DNAFTLHGLWPDKCSG--AYAPSGGCDSNRASSSIASVIKSKDSS-LYNSMLTYWPSNQG
T2 SDSWTIHGLWPDNCDG--SYGQF--CDKSREYSNITAILQEQGRTELLSYMKKYWPNYEG
121
140
160
180
S2 TKFDSLDKQAFWKDEYVKHGTC-------CSDKF----DREQYFDLAMTLRDKFDLLSSL
S3 ARADCIEHQYFWKHEYNKHGTC-------CSKSY----NLTQYFDLAMALKDKFDLLTSL
S6 EKADCMKTQNFWRREYIKHGTC-------CSEIY----NQVQYFRLAMALKDKFDLLTSL
RH ------NNNVFWSHEWSKHGTCVSTYDPDCYDNYEEGEDIVDYFQKAMDLRSQYNVYKAF
T2 ------DDEEFWEHEWNKHGTCINTIEPSCYKDYSPQKEVGDYLQKTVDLFKGLDSYKAL
181
200
220
240
S2 RNHGISRGFS--YTVQNLNNTIKAITGGF-PNLTCSRLR-ELKEIGICF----DETVKNV
S3 RKHGIIPGNS--YTVQKINSTIKAITQGY-RNLSCTKRQMELLEIGICF----DSKVKNV
S6 KNHGIIRGYK--YTVQKINNTIKTVTKGY-PNLSCTKGQ-ELNEVGICF----DSTAKNV
RH SSNGITPGG--TYTATEMQSAIESYF-GAKAKIDCSSGT--LSDVALYFY-VRGR---DT
T2 AKAGIVPDSSKTYKRSEIESALAAIHDGKKPYISCEDGA--LNEI-WYFYNIKGNAITGE
241
260
S2 IDCPNPKTCK-PTNKGVMFP------
S3 IDCPHPKTCKPMGHRGIKFP------
S6 IDCPNPKTCKTASNQGIMFP------
RH YVITDALSTGSCS-GDVEYPTK----
T2 YQPIDTLTSPGCSTSGIKYLPKKSEN

FIG. 1 Alignment of the amino-acid sequences (single-letter code) of the *S*-glycoproteins[6] with those of the ribonucleases Rh (ref. 4, and F.S., unpublished results) and T_2 (ref. 5) were made using the program CLUSTAL[11]. Gaps (-) have been introduced to optimize the alignment. The presumptive active-site histidine residues are marked by an asterisk. Boxed residues are identical among all five sequences, whereas similar residues (those belonging to the groups Asp, Glu, Asn and Gln (DENQ), Ser and Thr (ST), Ile, Leu, Met and Val (ILMV), and Phe, Trp and Thr (FWY) are marked by a circle (○). The amino-acid sequences of the S_2-, S_3- and S_6-glycoproteins of *N. alata* are from Anderson *et al.*[3]. The sequences of the ribonucleases Rh of *R. niveus* and T_2 of *A. oryzae* are from Horiuchi *et al.*[5] and Kawata *et al.*[4], respectively. The significance of the alignments was assessed with the ALIGN program[12]. With the mutation data matrix (250 PAMs), a bias of +6, and a gap penalty of 9, the alignments of the *S*-glycoprotein sequences with the RNase sequences had scores of 7.9–10.6 s.d. above the mean scores from 100 alignments of the randomized sequences. For comparison, the alignments of the *S*-glycoprotein sequences with the same parameters, had scores of 35.0–41.3 s.d. units. The alignment of the T_2 and Rh RNases gave a score of 21.8 s.d. units.

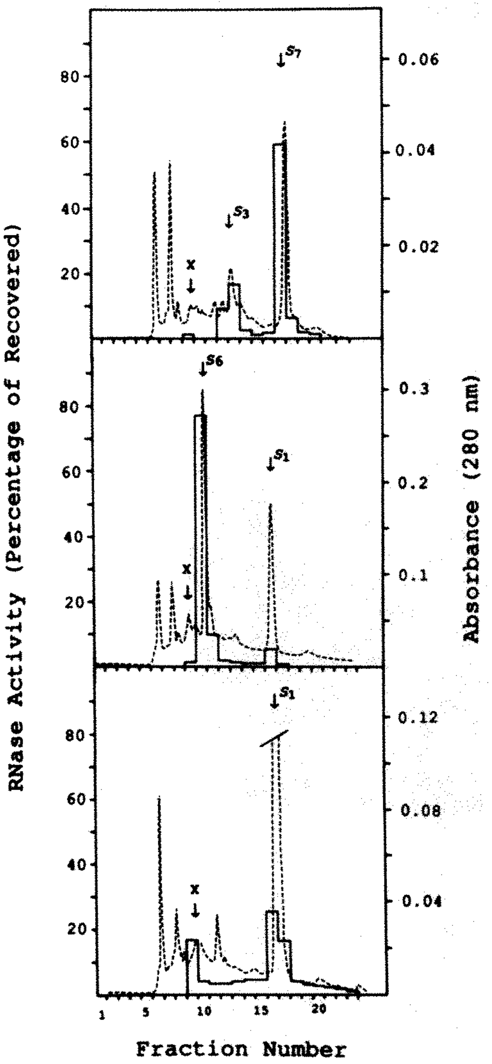

FIG. 2 Elution profiles of style extracts from mature flowers of *N. alata*. Extracts from styles of plants homozygous for the S_1-, S_3-, S_6- and S_7-alleles were prepared and fractionated by cation-exchange chromatography as previously described[6]. The elution profile was continuously monitored for absorbance at 280 nm (dotted line) and each fraction was assayed for ribonuclease activity[8] (solid line). *a*, Fractionation of an extract of a mixture of five styles, each of flowers homozygous for the S_3- and S_7-alleles. *b*, Fractionation of an extract of a mixture of 10 styles each of flowers homozygous for the S_6- and S_1-alleles. *c*, Fractionation of an extract from 20 styles of flowers homozygous for the S_1-allele. The elution position of each of the *S*-glycoproteins[6] is indicated. In each case, a major peak of ribonuclease activity co-fractionated with the *S*-glycoproteins on chromatography. A second peak of ribonuclease activity, not associated with the *S*-glycoproteins, is indicated by X. The ammonium-sulphate fractionation and desalting steps seem to cause no more than a 20% loss of activity. Thus 80% of the RNase activity in crude style extracts was subjected to cation-exchange chromatography. Total recovered activities were 64, 172 and 43 units in *a*, *b* and *c*, respectively. Conditions for isolation of the glycoproteins was as published[6] without further optimization for recovery of enzyme activity.

METHODS. Styles from defined *S*-genotypes of *N. alata*[2] were collected from plants maintained in the glasshouse. Extracts were prepared by homogenization (200 mg ml^{-1}) in 100 mM Tris-HCl butter, pH 7.8, 14 mM 2-mercaptoethanol containing 20 mg ml^{-1} Polyclar AT and 20 mg ml^{-1} Dowex 1×2 Cl (ref. 6). *S*-glycoprotein recovered from the extract after ammonium-sulphate fractionation (50–95%) was desalted into 50 mM sodium acetate, pH 5, by passage over a Sephadex G-25 column (Pharmacia) and applied to a Mono-S (HR 5/5) cation-exchange (Pharmacia) column. The column was developed with a linear NaCl gradient (0–500 mM at 1 ml min^{-1}) at room temperature (~18 °C). The eluate was continuously monitored for absorbance at 280 nm (dotted line). An aliquot of each 1-ml fraction was assayed for RNase activity essentially as previously described[8], except that the reactions were carried out in 100 mM imidizole-HCl, pH 7, 50 mM KCl at 37 °C for 30 min using torula yeast RNA (4 mg ml^{-1}; Sigma) as substrate. To reduce endogenous RNase activity, the substrate was repeatedly extracted with phenol:chloroform:isoamyl alcohol (25:24:1)[13], subjected to LiCl precipitation[14] and finally precipitated as the ammonium salt.

inhibition of the style RNase by the gametophyte.

This mechanism of inhibition of pollen tube growth is compatible with some observations on unilateral interspecific incompatibility[1]. One type of unilateral incompatibility is exemplified by reciprocal crosses between the self-incompatible species *N. alata* and the self-compatible species *N. tabacum*. Styles of all *S*-genotypes of *N. alata* have high RNase activity (Table 1) and support only limited growth of pollen tubes from *N. tabacum*. By contrast, styles from *N. tabacum* allow growth of pollen tubes from all *S*-genotypes of *N. alata*, leading to seed-set. It is significant that styles from *N. tabacum* do not contain an *S*-glycoprotein-like component and have relatively low total RNase activity (Table 1). The high level of RNase associated with the style *S*-glycoproteins could contribute to the rejection of *N. tabacum* pollen by mature *N. alata* styles (that is *N. tabacum* pollen could lack the ability to reject or inhibit the *S*-glycoprotein RNase). Conversely, *N. tabacum* could accept *N. alata* pollen by virtue of the absence of style *S*-glycoprotein RNase. Preliminary findings consistent with this view are that another gametophytic self-incompatible plant within the solanaceae *Lycopersicon peruvianum* has a high level of style RNase, whereas a closely related self-compatible accession has a low level of RNase activity (I. Lewis and B.A.M.-C., unpublished results). Also, bovine pancreatic RNase A is a nonspecific inhibitor of *in vitro* pollen tube growth, indicating that pollen tubes are susceptible to extracellular RNases (M. Lush, unpublished observation).

The results described here demonstrate that the *S*-glycoproteins have inherent RNase activity and indicate that this activity is involved in the inhibition of pollen tube growth in the self-incompatibility reaction of *N. alata*. This re-orientates our thinking about the function of the *S*-glycoproteins away from the traditional receptor–ligand model[1] to models based on cytotoxic enzyme activity. Indeed, these observations lead to a more generalized hypothesis for inhibition of pollen tube growth based on uptake by the gametophyte of a cytotoxic agent secreted by the style. In this model, the pollen *S*-gene product would enable non-self pollen to reject or inactivate the style cytotoxin, whereas pollen lacking an *S*-allele or bearing an *S*-allele identical to one present in the style would be unable to perform this function. ☐

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1. de Nettancourt, D. in *Monographs on Theoretical and Applied Genetics* (eds Frankel, R., Gall, G. A. E. & Linskens, H. F.) (Springer, Berlin, 1977).
2. Anderson, M. A. *et al. Nature* **321**, 38–44 (1986).
3. Anderson, M. A. *et al. Pl. Cell* **1**, 483–491 (1989).
4. Kawata, Y. *et al. Eur. J. Biochem.* **176**, 683–697 (1988).
5. Horiuchi, H. *et al. J. Biochem.* **103**, 408–418 (1988).
6. Jahnen, W. *et al. Pl. Cell* **1**, 493–499 (1989).
7. Jahnen, W. *et al. Pl. Cell* **1**, 501–510 (1989).
8. Brown, P. B. & Ho, T. H. D. *Pl. Physiol.* **82**, 801–806 (1986).
9. Cornish, E. C. *et al. Nature* **326**, 99–102 (1987).
10. Bradford, M. M. *Analyt. Biochem.* **72**, 248–254 (1976).
11. Higgins, D. G. & Sharp, P. M. *Gene* **73**, 237–244 (1988).
12. Dayhoff, M. O. *Meth. Enzym.* **91**, 524–545 (1983).
13. Maniatis, T., Fritsch, E. F. & Sambrook, J. *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, New York, 1982).
14. Wallace D. M. *Meth. Enzym.* **154**, 41–48 (1987).

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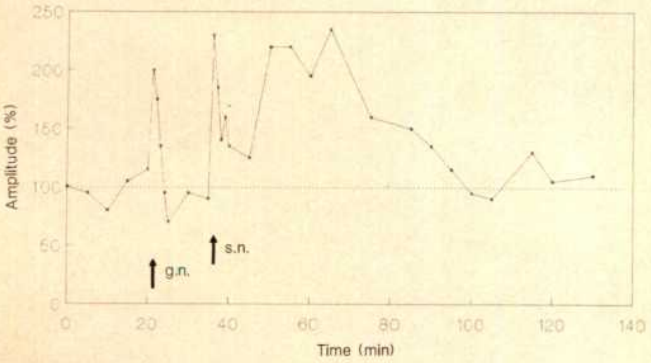
ERRATUM

Changes in spinal cord reflexes after cross-anastomosis of cutaneous and muscle nerves in the adult rat

Stephen B. McMahon & Patrick D. Wall

Nature **342**, 272–274 (1989)

OWING to editorial errors, an incorrectly labelled version of Fig. 2 was used in this manuscript, with the labels for the sural nerve (s.n.) and gastrocnemius nerve (g.n.) transposed. The figure is shown in its correct form below. ☐



CORRECTIONS

An optical yield that increases with temperature in a photochemically induced enantiomeric isomerization

Yoshihisa Inoue, Taizo Yokoyama, Noritsugu Yamasaki & Akira Tai

Nature **341**, 225–226 (1989)

IT has come to *Nature's* attention that this paper substantially duplicates a paper from the same authors in the *Journal of the American Chemical Society* **111**, 6480–6482 (1989). *Nature* was not made aware of the latter paper by the authors although it was submitted first. ☐

The winter solstice phenomenon at Newgrange, Ireland: accident or design?

T. P. Ray

Nature **337**, 343 (1989)

OWING to circumstances beyond *Nature*'s control, cooperation on the above project, an account of which appeared in our issue of 26 January 1989, was not acknowledged. Professor T. P. Ray would now like to correct this omission by stating that the name of T. M. O'Brien, consultant orthopaedic surgeon in Dublin, should be added to his own. Both were simultaneously engaged on research at Newgrange. Mr. O'Brien undertook the photographic and architectural surveys, Professor Ray the astronomical calculations of the Sun's path into the chamber at the solstitial winter sunrise. ☐

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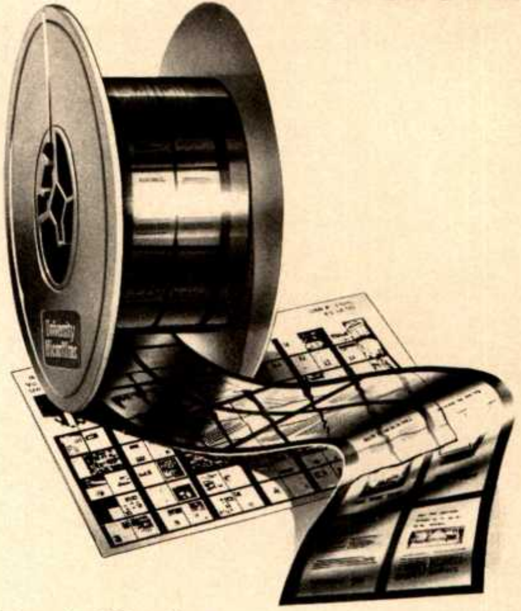

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The Department of Biology of the University of California, San Diego, invites applications for a tenure-track assistant professor position in mammalian molecular biology, animal virology or related areas.

Salary will be commensurate with level of appointment and based on the UC published salary scale. The successful candidate is expected to develop a vigorous research program in one of these rapidly-moving areas and participate in the department's undergraduate and graduate teaching program. A Ph.D. and several years of productive postdoctoral research training are required.

Please submit curriculum vitae, a statement of professional goals and research interests, and names of three references by February 15, 1990.

Mammalian Search Committee,
c/o Janet Brucker,
Department of Biology B-046,
University of California, San Diego,
La Jolla, CA 92093.

1868

The University of California, San Diego, is an equal opportunity/affirmative action employer. (NW4742)A

DSIR

DEPARTMENT OF SCIENTIFIC AND INDUSTRIAL RESEARCH

DIRECTOR OF NEW DIVISION CONCERNED WITH LAND AND ECOLOGICAL SERVICES

As part of the restructuring of the DSIR into larger operating units, on 1 April 1989 the DSIR Divisions of Botany, Ecology and Land and Soil Sciences will be regrouped into one large land resources Division, as yet to be named.

The new Division will consist of some 230 staff presently located mainly in the Hutt Valley, Christchurch and Palmerston North as well as at a number of smaller offices. The annual budget is presently $17.8M. The Division will undertake research and consultancy on land and soil characteristics and processes, botany and ecology and the application of this knowledge to practical problems, both in New Zealand and overseas.

The Director will be located in the Wellington area and will be responsible to the Director-General for all aspects of the management of the Division and for ensuring that the DSIR provides the Government with timely and effective scientific advice on matters concerning soil and land.

The successful applicant will have high qualifications and scientific attainment in land and soil science. Demonstrated leadership qualities with good administrative and financial management skills at a senior level are essential.

The conditions of employment and a substantial remuneration package commensurate with the importance of the position will be negotiated with the successful applicant.

Enquiries should be addressed:

Dr M.E.U. Taylor, DSIR, Head Office,
P O Box 1578, Wellington, NEW ZEALAND

Application should be made to the Director-General DSIR with a full curriculum vitae. Closing date for applications is 13 January 1990.

(W6853)A

Experimental Nuclear Physicist

There is a vacancy for an experimental physicist to join the Nuclear Physics Division. The experimental group is involved in carrying out and supporting a broadly based research programme using a 20MV Tandem.

The successful applicant will be expected to engage in research in nuclear physics and to play an active role in initiating and developing new programmes. Other duties will involve work on the design and development of major equipment through to commissioning, operation and maintenance. As a member of the in-house team of scientists he/she will be expected to provide expertise in the methods of experimental nuclear physics and to aid and direct the technical and scientific support staff in operating the Nuclear Structure Facility and for diagnosing problems as they arise.

Applicants should have a good honours degree in an appropriate discipline or equivalent with a period of postgraduate experience. A PhD degree in nuclear physics and a period of post doctoral experience in experimental nuclear physics would be an advantage. The appointment will be in the grade of Higher Scientific Officer with a salary range of £10,678 to £14,909, starting salary depending on qualifications and experience. Further increments are available depending on performance. The superannuation scheme is non-contributory.

CLOSING DATE: 12 January 1990

For further information please contact Dr. J. S. Lilley on (0925) 603558.

Application forms may be obtained quoting reference DL/141 from: The Personnel Officer, Science and Engineering Research Council, Daresbury, Warrington, WA4 4AD. Tel: (0925) 603467 (24 hour answering service).

(2356)A

Daresbury
SCIENCE & ENGINEERING RESEARCH COUNCIL

RESEARCH OPPORTUNITIES

The Commonwealth Scientific and Industrial Research Organisation (CSIRO) is a government research body with over 7000 staff, divided into 32 Divisions, each addressing specific industry sectors. The Division of Geomechanics is Australia's largest geomechanics research group, and undertakes research to increase the productivity, safety, and international competitiveness of the Australian mining, hydrocarbon, and civil engineering industries. The Divisional HQ is being located in new laboratories in Brisbane, Queensland, Australia.

RESEARCH ENGINEER

(Geotechnical)

To lead a multidisciplinary team developing new geotechnical applications in surface and underground coal mines and tunnels. Activities include new geomechanical analyses, development and implementation of field instrumentation, data analyses, laboratory testing and modelling for a variety of geotechnical projects.

RESEARCH GEOLOGIST

(Structural Modelling)

To study geological factors controlling the formation and exploitation of commercial coal seam methane deposits, and techniques to identify exploration targets. Development of 3D modelling techniques for the geology, structure, mechanical, and hydrological properties of exploration and production sites, in a graphic workstation/supercomputer environment.

RESEARCH ENGINEER/GEOLOGIST

(**In situ** Mining Technology)

To implement and lead research and development projects in **in situ** solution mining, in collaboration with mining companies and other interested parties. Research areas could include the hydrology of ore bodies, fluid flow/solution characteristics of ore, mechanics of **in situ** leaching, and enhancement of leaching performance.

RESEARCH ENGINEER

(Reservoir Engineering — Well Testing)

To undertake development of new well testing tools for the evaluation of the multi-phase (gas - water) flow characteristics of coal seams, and, as part of a skilled team, to organise and supervise industrial applications of new well testing technology - including data analysis.

Qualifications: PhD or equivalent in engineering, geology or related fields.

Tenure: Indefinite, with Australian Government superannuation benefits.

Salary: $A31525 to $53621

Further information: Including job descriptions and selection criteria, can be obtained by telephoning Mrs Elizabeth Mills in the London Office of the Agent-General for Queensland on(1)836 1333.

Applications: Quoting the names of at least two professional referees, should be sent to:

The Officer in Charge
CSIRO Division of Geomechanics
PO Box 63
St Lucia Qld 4067
Australia
by 19 January 1990.

(W6851)A

CSIRO
AUSTRALIA

CSIRO IS AN EQUAL OPPORTUNITY EMPLOYER

AB1 GM2

THE LONDON HOSPITAL MEDICAL COLLEGE
(University of London)

LABORATORY SCIENTIFIC OFFICER

Laboratory Scientific Officer required for the Dental Materials Laboratory of the London Hospital Medical College, to assist in the general running of the research laboratories and to participate in research projects. The research is largely on polymer science and technology applied to dental and other biomaterials; hence experience in this area would be advantageous.

Further details from Professor M Braden (Tel. 01-377 7619).

Salary scale £7,415-£10,148 plus £1,267 London Allowance per annum.

Applications to the **Secretary, The London Hospital Medical College, Turner Street, London E1 2AD.**

(2342)A

MERCK

We are a large corporation in the chemical-pharmaceutical industry, and are active in many countries all over the world. Our wide range of products is the result of our own research activities using state-of-the-art facilities.

For new developments in our pharmaceutical research we are looking for a qualified

Chemist or biochemist (Ph.D.)

who shall assume responsibility for our **department of immunochemistry**.

Requirements include a high degree of competence documented by several years' experience in the field of tumour-associated antigens as well as leadership qualities. The range of tasks for which the candidate shall be responsible are of ever increasing importance for our company and include the purification, characterization, and derivatization of monoclonal antibodies; in addition, the candidate should be capable of steering cell-culture techniques and molecular-biological projects. We are looking for a creative expert capable of developing and expanding this area of research on his/her own initiative. Dr. Irmscher, head of the preclinical pharmaceutical research division (Tel.: 06151/722636) will be glad to answer any questions you may have. Please send your application to

E. Merck
Personnel Department AT
P.O. Box 4119
D - 6100 Darmstadt
West Germany

(W6855)A

ROYAL FREE HOSPITAL

ACADEMIC DEPARTMENT OF MEDICINE

PRINCIPAL BIOCHEMIST

Salary: £16,760–£22,569 inc London Weighting

Applications are invited for the full-time permanent post of Principal Biochemist in the Academic Department of Medicine. The successful candidate should have research interests which suitably complement the strength of the Department, some of which are: i) Metabolism and nutrition in liver disease; ii) Alcohol abuse; iii) Iron metabolism and its relationship to liver disease (Haemochromatosis); iv) Lipoprotein metabolism; v) Molecular biology of viral hepatitis. Preference may be given to candidates with interest in application of biochemistry to research in liver disease.

For further information please contact Professor McIntryre on 01-794 0500 ext 3969.

Application forms and job description available from the Personnel Department, The Royal Free Hospital, Pond Street, London NW3 2QG; tel 01-794 0500 ext 4286 quoting reference number 765.

Closing date: 11th January 1990.

Hampstead Health Authority is an equal opportunities employer.

(2358)A

RCC (UK) LTD

RCC (UK) Ltd is part of an international contract research company with it's headquarters near Basle in Switzerland.

The main function of RCC (UK) Ltd is the histopathological evaluation of toxicity studies and we are now looking for a

TOXICOLOGICAL PATHOLOGIST

to work in our recently completed facility near Hereford.

Wide experience in the field is essential and an appropriate higher qualification would be an advantage. A high degree of independence is required and the appointee would be expected to maintain an interest in current developments in toxicological pathology.

An appropriate remuneration package is negotiable.

Please contact **Dr C J Springall**
RCC (UK) Limited
Willow Court
Netherwood Road
HEREFORD, HR2 6JU
Telephone: 0432 – 355176

(2355)A

UNIVERSITÄT KONSTANZ

At the Limnological Institute within the Faculty of Biology at the University of Konstanz the position of

Professor of Limnology

(full professor C-4, successor to Prof. Dr. Norbert Pfennig) is to be filled by October 1st 1990. It is desirable that the candidate in his (her) research combines field and laboratory investigations of microbial processes within inland water ecosystems. The teaching obligations will also apply to the University of Freiburg. It is expected that the new holder of the professorship participates in the Special Collaborative Programme (SFB) 248 "Cycling of Matter in Lake Constance" and the graduate course on "Ecology" to be newly installed at the University of Konstanz.

Preference will be given to physically handicapped people with equivalent qualifications.

Applications are to be directed by **15 January 1990** at the latest to **Dekanat der Fakultät für Biologie der Universität Konstanz, Postfach 55 60 D-7750 Konstanz**, where also further information can be obtained. (W6845)A

An der Naturwissenschaftlichen Fakultät der Universität Salzburg ist die Planstelle eines(r)

ORDENTLICHEN UNIVERSITÄTSPROFESSORS(S)/-IN FÜR MOLEKULARE GENETIK

zu besetzen. Von den Bewerbern wird die Vertretung dieses Faches in Lehre und Forschung im Rahmen der Studienrichtung Biologie, insbesondere des Studienzweiges Genetik, der Aufbau einer Forschungsgruppe und die Zusammenarbeit mit anderen Universitätsinstituten und dem Akademieinstitut für Molekularbiologie erwartet. Bewerbungen sind bis 28. Februar 1990 an den Dekan der Naturwissenchaftlichen Fakultät der Universität Salzburg, O.Univ.-Prof. Dr. Georg AMTHAUER, Hellbrunnerstrasse 34, A-5020 Salzburg, zu senden.

(W6863)A

UNIVERSITY COLLEGE AND MIDDLESEX SCHOOL OF MEDICINE

UCL

UCMSM

ACADEMIC DEPARTMENT OF PSYCHIATRY AND MEDICAL MOLECULAR BIOLOGY UNIT

RESEARCH ASSISTANT or SCIENTIFIC OFFICER in HUMAN MOLECULAR GENETICS

To join a research group employing molecular genetic techniques in a variety of diseases. The position is funded by the MRC human genome mapping programme for the development of research to identify coding sequences by the use of oligonucleotide hybridisation. The position is for two years in the first instance and is suitable for M.Phil./Ph.D. registration. Salary will be on the research assistant 1B (predoctoral) or scientific officer scales. For further information ring or write as soon as possible to **Dr. H. Gurling, Tel. 01-380-9474/9475, or Dr. G. Melmer on 01-636-8333 exts. 3073 or 3311 at the Academic Department of Psychiatry, University College and Middlesex School of Medicine, Riding House Street, London W1P 7PN.** Applicants should send a full C.V. and the names of at least two referees.

(2345)A

（株）アイ・シー・オー

薬事申請関連総合ドキュメンテーション

翻訳者（在宅）及びアドバイザー（在宅）募集

▶**職　務**：基礎（薬理・毒性・ADME等）及び臨床の翻訳、並びに薬事申請関連ドキュメンテーションのコンサルタント業務

▶**資　格**：医薬・農薬・動薬・化粧品・食品・医学に関する知識と語学力を有する方
年齢・性別不問

▶**待　遇**：出来高制

▶**応　募**：履歴書郵送又は持参　※応募秘密厳守

▶**連絡先**：〒152 東京都目黒区鷹番3-6-9-405
〈担当：今〉 TEL(03)712-2377
FAX(03)712-2414

大阪支店 〒541 大阪市中央区南久宝寺町4-5-11-606
〈担当：馬渕〉 TEL(06)243-5980
FAX(06)243-5981

(W6866)A

DEPARTMENT OF BIOCHEMISTRY
THE UNIVERSITY OF ARIZONA

FACULTY POSITION IN X-RAY CRYSTALLOGRAPHY

Applications are invited for a tenure-track position in x-ray crystallography for macromolecular systems. Although there is a preference for candidates at the Assistant Professor level, applicants at more senior levels will be considered. This is part of an ongoing effort to develop an exceptionally strong program in structural biology and biophysics. We have recently added three NMR spectroscopists and an x-ray crystallographer to this program, and plan to fill additional positions in the next 1-2 years. Applicants will be expected to carry out a vigorous research program with ample opportunities for collaboration, as well as to contribute to our graduate and undergraduate teaching program. An excellent crystallographic facility is available, with fund in place for the purchase of additional equipment. Applicants should submit curriculum vitae, representative reprints, a short statement of research plans, and have at least three letters of reference sent to: **Dr. William Montfort, Co-chair, Search Committee, Department of Biochemistry, Biosciences West, The University of Arizona, Tucson, AZ 85721.**

Review of applications will begin January 15, 1990, but the position will remain open until filled.

Women and minorities are urged to apply.

An Equal Opportunity/Affirmative Action/Title IX/ Section 504 Employer.

(NW4762)A

THE UNIVERSITY OF CALIFORNIA AT SAN DIEGO
DEPARTMENT OF BIOLOGY

FACULTY POSITION IN CELL BIOLOGY

The Department of Biology of the University of California, San Diego, invites applications for a tenure-track assistant professor position in cell biology. Our department covers a broad range of topics including cellular biology, molecular biology, neurobiology, and developmental biology. We are seeking outstanding candidates in the field of cellular biology with emphasis in one of the following areas:

- protein sorting
- signal transduction
- cell-cell interactions
- extracellular matrix
- cell motility

Exceptional applicants in all areas of cellular biology will be considered. Salary will be commensurate with level of appointment and based on UC published salary scale. The successful candidate is expected to develop a vigorous research program and participate in the department's undergraduate and graduate teaching program. *We strongly encourage women and minorities to apply.* Please submit curriculum vitae, names of three references, reprints of publications, and a statement of present and future research plans by February 15, 1990.

Cell Search Committee,
c/o Lynn Grimes,
Department of Biology B-046,
University of California, San Diego,
La Jolla, CA 92093.

The University of California is an Affirmative Action/Equal Opportunity Employer.

(NW4743)A

UNIVERSITY OF ZIMBABWE

Applications are invited for the following posts:

PROFESSORSHIP

Department of Biological Sciences

Applicants should be experienced scientists of established international reputation in any field of plant science involving relevant areas of microbiology, ecology and genetics.

ASSOCIATE PROFESSORSHIPS/SENIOR LECTURESHIPS/LECTURESHIPS

Department of Biological Sciences

A Biomathematician is required to teach basic statistical procedures and computer usage.

Department of Geology

Applications are sought from economic geologists with practical experience of mineral exploration and/or mining geology.

CONDITIONS OF SERVICE

Salary Scales: Lecturer: Z$22,488–Z$31,692; Senior Lecturer: Z$32,736–Z$35,436; Associate Professor: Z$36,492–Z$37,680; Professor: Z$38,064 (fixed). Appointment on the above scales will be according to qualification and experience.

Both permanent and short-term contracts are offered. Persons who are not Zimbabwean citizens may be appointed only on a short-term contract basis for an initial period of two years. Short-term contracts may be extended. Applicants should quote reference number ASA/16/11/89 and submit six copies of applications giving full personal particulars which should include full name, place and date of birth, qualifications, employment and experience, present salary, date of availability, telephone number and names and addresses of three referees to the Deputy Registrar (Administration), University of Zimbabwe, P O Box MP 167, Mount Pleasant, Harare, Zimbabwe. Telegrams: UNIVERSITY; Telex: 4–152 ZW. Applicants from outside Zimbabwe should also send a copy to the **Appointments Officer, Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF, UK**, from whom further details may be obtained.

Closing date for receipt of applications is 18 January 1990.

(W6843)A

TENURE-TRACK FACULTY POSITION

UCR/UCLA PROGRAM IN BIOMEDICAL SCIENCES

CELL BIOLOGY/HISTOLOGY

The UCR/UCLA Program in Biomedical Sciences, which offers an accelerated M.D. curriculum jointly between the University of California, Riverside (UCR) and the University of California, Los Angeles (UCLA) School of Medicine, has a tenure-track faculty position open in the Division of Biomedical Sciences at UCR available July 1, 1990.

Primary responsibilities will be one-half of a 12-week lecture and laboratory course in histology for 24 first-year medical students. Prior medical school teaching experience is strongly preferred. The successful candidate will also be expected to actively participate in graduate student training.

The committee welcomes applications from those with research interests in any area of cell biology who would enjoy working within a multidisciplinary environment.

The prospective level of appointment for candidates, who must possess the Ph.D. and/or M.D. degree, may be at the Assistant, Associate, or Full Professor level. Junior applicants must have postdoctoral research experience and the potential to develop a strong, extramurally funded research program. Senior applicants should have a clearly demonstrable record of research productivity, success in obtaining extramural funding, and demonstrated teaching effectiveness. The University of California is an Affirmative Action/Equal Opportunity employer. The deadline for application is February 15, 1990. Applicants should send a curriculum vitae, representative reprints, a statement of present and future research goals, summary of teaching experience, and arrange to have three letters of recommendation sent to:

Dr Ameae M. Walker
Cell Biology/Histology Search Committee
Division of Biomedical Sciences
University of California
Riverside, California 92521-0121

(NW4744)A

PALEOECOLOGY OF TROPICAL AMERICA

SMITHSONIAN TROPICAL RESEARCH INSTITUTE

The Smithsonian Tropical Research Institute is planning a major expansion of its terrestrial and marine research program in the paleoecology of tropical America. The Institute is a bureau of the Smithsonian Institution and has headquarters in the Republic of Panama. The paleoecology program is documenting the environmental and ecological history of the region over the last 5 million years. We anticipate two new permanent staff positions to begin in October 1990. We invite applicants at any level in the following disciplines: vertebrate paleontology, paleobotany, invertebrate paleontology, micropaleontology, paleooceanography, and paleoclimatology. Annual salary from $30,000 to $59,000 plus benefits and allowances. Level will depend on qualifications. Substantial set-up funds and research support. Screening will begin March 15, 1990 and continue until positions are filled. Applications should include statement of research interests, curriculum vitae, reprints, and names, addresses, and telephone numbers of 5 references. Send to:

Paleoecology Search Committee
Smithsonian Tropical Research Institute
APO Miami 34002-0011, U.S.A.

The Smithsonian Institution is an Equal Opportunity/Affirmative Action Employer, and appointment can be made without regard to nationality. (NW4750)A

RESEARCH ASSISTANT PROFESSOR

The Department of Physiology at Temple University School of Medicine invites applications for a non-tenure track position at the level of Research Assistant Professor. Ph.D. in Physiology and 1–2 years post-doctoral training required. Candidate must have laboratory experience in experimental physiology, protein biochemistry, and handling of radioactive materials. Applicant will be expected to conduct a variety of experiments involving in vivo thrombosis models, which require microvascular surgery. Skill in ligand-receptor binding studies, polyclonal antibody production, human venopuncture, radiolabeling of proteins and protein purification also beneficial. Primary research area will involve the purification of various snake venom peptides and the evaluation of their potential antithrombotic activity in biological systems. Opportunity also available for participation in research focused on the localization of specific binding epitopes for plasma proteins on platelet membrane glycoproteins.

Responsibilities will include the coordination of laboratory activities and the supervision of students and technical personnel as well as independent and collaborative research. Applicant must present evidence of excellent verbal and written communication skills and the ability to obtain research funding. Please send curriculum vitae and three letters of reference to: **Dr. Peter R. Lynch, Chairman,**

Temple University
School of Medicine
Department of Physiology
3420 N. Broad Street
PHILADELPHIA, PA
19140

(NW4715)A

Faculty Positions Memorial Sloan-Kettering Cancer Center Cell Biology and Genetics Program

The Memorial Sloan-Kettering Cancer Center invites applications for several tenure-track junior and senior faculty positions in the Cell Biology and Genetics Program. This research program is one of four in the institute, and is expanding in the areas of cell cycle control, differentiation, cell structure, intercellular recognition, and morphogenesis. The Cell Biology and Genetics Program is located in a new research building which also houses advanced facilities for protein and nucleic acid microchemistry, transgenic mice, and automated mammalian cell micromanipulation. The members of the Cell Biology and Genetics program faculty are affiliated with the Cell Biology Graduate Program at Cornell University Medical College. The positions offered are highly competitive with regard to start-up funds, laboratory space, salary and housing.

Eligible candidates should have a doctoral degree, postdoctoral experience and demonstrate potential and ability to develop an independent, imaginative research program. Formal applications are invited from junior as well as established investigators. Applications should include a curriculum vitae, a list of publications, a summary of research activities and research plan and three letters of recommendation. Applications should be addressed to: **Dr Joan Massague, c/o Christine Brohme, Box # 135, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, N.Y. 10021.**

Equal employment opportunity/
Affirmative Action

(NW4481)A

POST DOCTORAL RESEARCH ASSOCIATES

Several positions are availab
to work on eicosanoids, gonad
tropins and growth factors reg
lation of reproductive endocri
functions.

Candidates with experience
the following areas will be co
sidered: cell micromanipulatio
techniques, inositol phospholip
metabolism, protein kinase
Ca^{2+} fluxes, eicosanoids synth
sizing enzmes, smooth musc
contractions, etc.

If interested, contact **Dr. Ch.**
Rao, Dept. of Ob/Gyn, 438 MD
Bldg., University of Louisvill
Louisville, KY 40292.

(NW4740)A

Molecular Biologis

Position open for a perso
preferably with a PhD degre
with molecular biology bac
ground. Research proje
includes demonstration of th
gene, and receptor expressio
and the role of cytokines (IL-
IFN-gamma, TNF and IL-1) a
well as their relation to se
steroid hormone action
human endometrium. Sen
CV to **Dr. Tabibzadeh, Dept**
Pathology, Elmhurst Hospita
79-01 Broadway, Elmhurst, N
11373 or call: 718-830-1685.

(NW4754)A

SIMON FRASER UNIVERSITY
CHAIR OF THE DEPARTMENT OF BIOLOGICAL SCIENCES

Applications and nominations are sought for the position of Chair of the Department of Biological Sciences at Simon Fraser University. The Department has 35 faculty members whose teaching and research interests cover all the modern areas of Biological Sciences. There is a graduate enrolment of some 160 students, and external research grant support in 1988/89 was $2.4 million.

A Departmental Chair appointment is for up to five years and is renewable; the salary is negotiable. The appointment would normally carry tenure. Candidates should have an excellent record in research and a demonstrated ability as an academic leader.

Applications or nominations should be forwarded, by January 15, 1990, to the **Dean of Science, Simon Fraser University, Burnaby, B.C. V5A 1S6.**

This advertisement is directed to people who are eligible for employment in Canada at the time of application. S.F.U. offers equal employment opportunities to qualified applicants. (NW4626)A

MOLECULAR IMMUNOLOGIST

The Department of Microbiology, Immunology and Parasitology, New York State College of Veterinary Medicine, Cornell University, seeks candidates for a senior faculty position in Immunology. Rank and salary will be commensurate with experience. The successful candidate will be expected to have an independent, competitively funded research program emphasizing molecular or cellular aspects of immunology. In addition, it is anticipated that she/he will assume a leadership role in immunology in the department, contribute to the teaching of the discipline, and have the opportunity to participate in a campus wide program in Immunology. Applicants should have a PhD, DVM/PhD or MD/PhD and several years' research experience. This position is part of a major commitment by the department to use molecular approaches in biomedical research. Applications including a curriculum vitae, outline of future research plans and the names and addresses of three referees should be sent to **Dr. Roger J. Avery, Chairman, Department of Microbiology, Immunology and Parasitology; College of Veterinary Medicine, 616 Veterinary Research Tower, Cornell University, Ithaca, NY 14853-6401** by February 15, 1990. Telephone inquiries will be welcomed (607/253-3400).

Cornell University is an equal opportunity/affirmative action employer/educator. (NW4738)A

ASSISTANT PROFESSOR

Applications are invited for a position at the level of assistant professor (tenure track) in the Department of Chemistry at Stanford University, with primary research and teaching interests in the general area of inorganic chemistry. Applicants must be strongly motivated toward creative research and have a commitment to teaching at the undergraduate and graduate levels. In applying, please send curriculum vitae, list of publications, a brief statement of research interests (three pages or less) and, in addition, have three letters of recommendation sent to **Professor Harden M. McConnell, Chairman, Department of Chemistry, Stanford University, Stanford, California 94305.**

Stanford is committed to Equal Opportunity through Affirmative Action in employment, and we are especially eager to identify minority persons and women with appropriate qualifications. Applications should be received by January 15, 1990. (NW4629)A

United Medical and Dental Schools of Guy's and St Thomas's Hospitals

RESEARCH ASSISTANT 1B

DIVISION OF PHYSIOLOGY

required in our St Thomas's Campus to use microepifluorescence techniques to study intracellular ions in isolated cells. Other duties involve preparation of cells for epifluorescent studies and day to day running of the laboratory. Candidates should have a good BSc degree. Appointment for a period of one year.

Salary range £9,816-£12,381 plus £1,650 LWA according to age, qualifications and experience. For a job description please contact Mrs F Diamond on 01-928 9292 ext. 3341.

Applications with full CV and names and addresses of two referees to the **Personnel Officer, UMDS, St Thomas's Campus, Lambeth Palace Road, London SE1 7EH** by 11 January 1990 quoting reference STH/PHYS/465. (2350)A

THE UNIVERSITY OF WOLLONGONG

P.O. BOX 1144, WOLLONGONG, N.S.W. 2500
PHONE (042) 27 0555 FAX (042) 27 0433 TELEX 29022
AUSTRALIA

The University of Wollongong has established a reputation as an energetic, innovative institution. It has grown rapidly in recent years and currently enrols some 8,000 students in seven faculties, Arts, Commerce, Education, Engineering, Health and Behavioural Sciences, Mathematical Sciences and Science.

Professor of Applied Mathematics

Applications are invited from outstanding scholars whose teaching and research activities will further enhance the reputation in Applied Mathematics of the University. The Department of Mathematics has an established research programme in "Engineering and Industrial Mathematics" which also includes members from the Faculty of Engineering. The appointee will be expected to take a leading role in this research programme through his or her own research, industrial collaboration and enterprise. The research and teaching of the Department covers the whole spectrum of Pure and Applied Mathematics and Statistics and the successful applicant will also be expected to contribute to the balanced development of these disciplines.

A restructuring will, from next year, place the Department in a Faculty of Informatics with the Department of Computing Science, Electrical and Computer Engineering and an Information Technology and Communication Unit. Further information from Professor David Griffiths 61 42 27 0845. Reference AC 89-80. Closing date 19 January, 1990.

Professors may, with approval, undertake a limited amount of consultative work. Appointment as Head of Department on a rotating basis is a requirement. The University reserves the right to fill an advertised Chair by invitation. Remuneration will be based on a salary of $A65,847 per annum and includes a flexible benefits package.

Initial inquiries may be made to Mr Ross Walker 61 42 27 0934. Applications should quote the Reference, contain details of qualifications, employment history, research interests, publications and the names and addresses of three referees and should be addressed to the Recruitment Officer (Academic Staff). A secure fax is available at 61 42 27 0700. Please mark envelope "Confidential-Appointment".

Equality of employment opportunity is University Policy. (W6852)A

POSTDOCTORAL POSITIONS IN IMMUNOLOGY AND MOLECULAR VIROLOGY

Two positions are available immediately to study apitopes of human immunodeficiency virus (HIV-1) recognized by cytotoxic T lymphocytes (CTL). Approaches being taken include the construction of recombinant viral vectors, cloning of human anti-HIV CTL and use of synthetic peptides. Experience in immunology or molecular biology/virology is desirable. Send curriculum vitae and the names of three references to: **Dr. Ken Rosenthal, Department of Pathology, McMaster University Health Sciences Centre, Hamilton, Ontario, Canada L8N 325.** (NW4759)A

POSITION AVAILABLE

RESEARCH ASSOCIATE PROFESSOR OF PHARMACOLOGY — To work on the isolation of insulin mediator and anti-mediator substances and elucidate their structure, mechanism of action and biosynthesis.

Candidates should have a strong chemical background in the isolation and identification of biological components knowledge of physico-chemical techniques such as mass spectrometry, mono spectroscopy would be an advantage. Competence and biological assay is essential. Applicant should be well informed in the mechanism of insulin action. Previous experience with insulin mediator would be an advantage.

Send Curriculum Vitae to **Dr. Joseph Larner, Department of Pharmacology, University of Virginia, Charlottesville, Virginia 22908.** An Equal Opportunity/Affirmative Action Employer. (NW4739)A

DEPARTMENT OF CHEMISTRY

The Department of Chemistry and Biochemistry at Concordia University, Montreal hopes to make a tenure-track appointment in Biochemistry to commence in August 1990. The Department has strong research interests in the interface of Chemistry and Biology in Bioinorganic Bioorganic and Biophysical Chemistry. We are seeking an outstanding candidate with research interests in areas of Basic Biochemistry, such as: Enzymology, Metabolism or Membrane Enzymes, that will compliment our existing interests. The successful applicant will have to have a Ph.D. and Post Doctoral experience, will be required to establish a vigorous Research Program that will attract external funding, and teach Biochemistry effectively at the Undergraduate and Graduate levels. Start-up assistance will be provided by the University, while external funds are available from Quebec and federally from the Natural Sciences and Engineering Research Council of Canada. The current salary floor for Assistant Professors is $33,278 per year.

Concordia University is a predominatly English Institution located in downtown Montreal close to three other Research Intensive Universities. There are 29 full-time Faculty in the Department of Chemistry and Biochemistry with active Research Program in Biochemistry, Organic, Inorganic and Physical Chemistry, Photochemistry, and Material Science. At the present time there are 50 Graduate Students enroled at the M.Sc. and Ph.D. levels in the Department and about 15 of these are doing Research in the area of Biochemistry. The Department is housed adjacent to the Biology Department and this facilitates collaborative Research interactions between Biochemists in the two Departments.

All qualified individuals are encouraged to apply, but in accordance with Canadian Immigration requirements, this Advertisement is primarily directed to Canadian citizens and permanent residents. Applicants should supply a Curriculum Vitae, a short Resume of Research interests, a summary of start-up requirements and arrange for three letters of reference to be sent to:

Dr. P.H. Bird
Department of Chemistry and Biochemistry
Concordia University
1455 de Maisonneuve Blvd., West,
Montreal, Quebec
H3G 1MB (NW4758)A

IMPERIAL COLLEGE OF SCIENCE, TECHNOLOGY AND MEDICINE

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH ASSISTANT IN NEUROSCIENCE

Applications are invited from persons possessing, or who will shortly possess, a PhD in the neurosciences for an appointment for up to three years, to work on the cell biology of demyelination and remyelination in models of Multiple Sclerosis. The successful candidate will join a group investigating the cell biology of CNS glial cells during normal and abnormal development, and their response to damage. Previous experience in tissue culture, immunohistochemistry and neuroimmunolgy would be particularly useful.

The post is tenable from 1st March 1990. Initial salary will be in the range £14,031 – £15,177, inclusive of London weighting, on the RA1A scale. Applications should be made in writing (full CV, publication list, summary of research to date, and names and addresses of two referees) to **Dr Richard Reynolds, Department of Biochemistry, Imperial College, Kensington, London SW7 2AZ.** Interested persons can call Dr. Reynolds on 01-589 5111 ex 4135. (2347)A

POSTDOCTORAL POSITION

Available immediately for a Ph.D. in immunology to study T cell recognition. The work will focus on the role of CD8 and CD4 molecules and will include both molecular and cellular investigations. Previous experience with cellular immunological and/or molecular biological techniques would be an advantage but is not essential.

Send CV, including names and tel. no.'s of 2 references to **Dr. Terry Potter, Division of Basic Immunology, Dept. of Medicine, National Jewish Center, 1400 Jackson St., Denver, CO 80206.** (NW4761)A

教 授 公 募

京都大学理学部生物物理学教室

1. 公募人員：教授１名
2. 所属講座：分子生物学講座
3. 研究分野：分子生物学（分子生物学における新しい領域を意欲的に開拓しようとする人を広く募集する。）
4. 提出書類：履歴書、論文リスト、主要論文別冊10編以内、従来の研究の概要と今後の研究・教育における抱負、意見を尋ねられる人の名前２名。
5. 自薦、他薦を問いません。他薦の場合は、必ずしも全部の書類を揃える必要はありません。
6. 公募締切：1990年３月末日
7. 提出先：〒606 京都市左京区北白川追分町、
   京都大学理学部生物物理学教室、主任　志村令郎

（封筒に分子生物学講座教授人事応募と朱書して下さい。）

(W6865)A

PHLS CENTRE FOR APPLIED MICROBIOLOGY AND RESEARCH

DIVISION OF PATHOLOGY

Senior Grade Microbiologist (Immunologist) 2 years, MRC.

To work on development and application of a primate model of human AIDS. This project is an essential part of the MRC AIDS-drected programme, as a model is needed for testing vaccine approaches and potential therapies and for studies of pathogenesis. The project is progressing well and offers unique opportunities for analysis of cell-mediated immune responses to infection. Advances in this area will be crucial to rational vaccine formulation.

The candidate for this post will ideally be post-doctoral, possessing a good understanding of cellular immunology and with research experience in this area, preferably in relation to virus infection. Experience in haematology or flow cytometry would also be valuable. However, applicants with research experience in areas such as virology or cell biology would be considered for the post.

The successful applicant will have the opportunity for contact with scientists working in AIDS research both at CAMR, where there is a large team engaged on a variety of projects, and within the MRC AIDS Directed Programme.

Salary will be on the scale of £11,778-£15,394 (under review).

National Health Service terms and conditions will apply.

This post may be discussed informally with Dr M Cranage or Dr T Ashworth on Ext 2317/2342.

Application forms can be obtained from the Personnel Officer, PHLS Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wilts SP4 0JG, Telephone Idmiston (0980) 610391 ext 240, to whom completed forms should be returned by 5th January 1990 quoting post number 1130. (2344)A

THE UNIVERSITY OF MELBOURNE

SENIOR LECTURER/ READER (EXTENDIBLE TENURI

MEAT SCIENCE

School of Agriculture
and Forestry

The University of Melbour Australia seeks applications fc Senior Lecturer or Reader Meat Science. This position within the Animal Science sect of the School of Agriculture Forestry, and has been fundec the Pig Research Council for years initially. The appointee be responsible for the devel ment of a strong research gramme in Meat Science particular reference to pig m Excellent opportunities exist collaboration with resea groups in the various s Departments of Agriculture the CSIRO, as well as teaching and supervision of p graduate students. Qualificati include a Ph.D. in Meat Scie or related discipline, with dem strated productivity in resear

Enquiries to Professor A. Egan phone (61 3) 344-5007, (61 3) 344-5104.

Salary is in the rar $A42,703-$A49,529 per anr (Senior Lecturer) and $A55, per annum (Reader).

Position Number: 206A030

This position will remain o until a suitable candidate found.

Written applications shc reach The Director, Persor Services as stated below.

Applications, in duplic including names and addre of at least three referees quoting the relevant posi number should be addressed The Director, Personnel Servi The University of Melbour Parkville, Victoria, 3(Australia.

An equal opportunity ployer. (W6850)

NATIONAL BLOOD TRANSFUSION SERVICE

CAMBRIDGE

pplications are invited for the ewly created post of Deputy QA fficer in the Quality Assurance epartment, which has been operting since July 1988 and is introucing a quality system.

The Deputy QA Officer will; ke part in quality audits of the ork of the Centre; be involved in uality monitoring and training of aff in GLP and GMP.

Potential applicants must have a .1 or first class honours degree in harmacy or an appropriated life ciences subject and will have had ractical experience of Quality Assurance.

They should ideally have worked in some aspect of Transfuion Science and be qualified in, or ave experience of, microbioloical techniques. However, the uccessful applicant will be given ppropriate training to overcome ny deficiencies which may exist n their previous practical xperience.

Informal visits are welcomed nd may be made by arrangement with the Director.

Applications stating age, qualifications, present post, previous experience and the names of two referees to the Director, Regional Transfusion and Immuno-Haematology Centre, Long Road, Cambridge. CB2 2PT.

Closing date for applications will be Wednesday 31st January 1990. (2340)A

WESSEX REGIONAL CYTOGENETICS UNIT

BASIC GRADE CYTOGENETICIST

A Basic Grade level position has become available within this Unit which provides a comprehensive diagnostic cytogenetic and molecular genetic service for the Wessex Region.

Applicants should have some experience of, or an interest in, clinical cytogenetics. A first or second class honours degree in a relevant subject is required. Salary range £8,255-£10,369 (under review). Further information may be obtained from Dr P A Jacobs or Dr Nina Gregson. Telephone Salisbury (0277) 336212 Ext. 669.

For an Application Form and Job Description please WRITE to the **Personnel Department, Odstock Hospital, Salisbury, Wiltshire SP2 8BJ.**

Closing date: 26th January 1990.

(2354)A

MURDOCH UNIVERSITY

Perth, Western Australia

Agreement has been reached between Murdoch University and the University of Western Australia to seek amalgamation, subject to legislative approval, of the two institutions by January 1990.

SCHOOL OF BIOLOGICAL AND ENVIRONMENTAL SCIENCES

NITROGEN FIXATION GROUP

POST-DOCTORAL RESEARCH FELLOWSHIPS (TWO)

The following posts are available in the Nitrogen Fixation Research Group to work with Associate Professor A R Glenn and Professor M J Dilworth on the physiology, biochemistry and molecular biology of *Rhizobium*.

Molecular Biologist
Applications are invited for a post-doctoral research fellowship funded by the Australian Wool Corporation to work on the genetics of acid-tolerance in the N-fixing organism *Rhizobium meliloti*. The post is available for 3 years. (REF: 1288)

Biochemist
Applications are invited for a post-doctoral research fellowship funded by the Australian Research Council to work on the characterization of malic enzyme and its genes in *Rhizobium leguminosarum*. The post is available for 2 years. (REF: 1299)

Further details can be obtained from Associate Professsor Andrew Glenn (09) 332 2231 fax (09) 310 3505.

Salary: A$27,953 per annum.

APPLICATIONS IN DUPLICATE quoting reference number, including full personal particulars, details of tertiary qualifications and experience, a curriculum vitae and academic transcript and the names and addresses of three professional referees should reach the Chief Personnel Officer, Murdoch University, Murdoch WA 6150 By *19 January 1990.*

Applicants resident in the United Kingdom, Europe or Africa, at the time of application should also forward ONE further copy to Appointments (37308), Association of Commonwealth Universities, 36 Gordon Square, London WC1H 0PF.

MURDOCH UNIVERSITY IS AN EQUAL OPPORTUNITY EMPLOYER

(W6846)E

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nature

Still first with the best in science

MAX-PLANCK INSTITUT FÜR IMMUNBIOLOGIE

POSTDOCTORAL MOLECULAR BIOLOGIST

Applications are invited from suitably qualified scientists to join an independent group studying aspects of the regulation of eukaryotic gene expression by growth factors.

The post is available from 1st August 1990, initially for a period of three years but with the possiblity of extension.

Remuneration will be in accordance with the German Civil Service Salary Scale (BAT).

Informal enquiries to: Peter Shaw, Zentrum für Molekulare Biologie der Universität Heidelberg. F.R.G. (Tel. 06221 566825).

Applications including curriculum vitae, list of publications and names and addresses of two referees should be sent to:

Personalabtellung,
Max-Planck-Institut für Immunbiologie,
7800 Freiburg, Postfach 1169, F.R.G.

(W6847)A

LEPIDOPTERAN BIOLOGY

HARVARD UNIVERSITY

DEPARTMENT OF ORGANISMIC AND EVOLUTIONARY BIOLOGY

THE SIDNEY A. AND JOHN H. HESSEL PROFESSORSHIP OF BIOLOGY, for research and teaching in lepidopteran biology, with responsibility for curating the lepidopteran collection of the Museum of Comparative Zoology.

Applicants should have demonstrated excellence in research and a commitment to teaching at the graduate and undergraduate levels as well as a strong interest in systematics and a readiness to assume curatorial responsibilities for a large and important collection of Lepidoptera. A curriculum vitae, statement of interest and names of three recommenders should be sent to the committee no later than January 22, 1990.

Committee: Hessel Professorship Search
(Attention E.O. Wilson)
Department of Organismic and Evolutionary Biology
26 Oxford Street
Cambridge, MA 02138-2902, U.S.A.

Harvard University is an Affirmative Action/Equal Opportunity Employer. (NW4757)A

Washington University, St. Louis

Postdoctoral – Research Associate Level

Applications are invited for two postdoctoral positions to investigate the cellular and molecular biology of bone development. A background in protein chemistry and/or molecular biology is desirable. Ongoing studies utilize cell culture and monoclonal antibodies to investigate cell surface molecules and factors influential in their expression. Positions offer competitive salaries and are available immediately. Curriculum Vitae and the names and addresses of two references should be sent to **Dr. Philip Osdoby, Department of Biomedical Sciences, Washington University, 4559 Scott Avenue, St. Louis, Mo. 63110.** (NW4748)A

Postdoctoral Position

Mammalian Oocyte Development

A postdoctoral position is available in the laboratory of Dr. John Eppig to study mammalian oocyte development. Emphasis will be in two areas (1) the initiation of oocyte maturation, and (2) the expression and role of the *Mos* proto-oncogene in oocyte development. Experience in techniques of molecular biology is desirable.

Candidates should be available between the Spring and Fall of 1990. Send a CV and a letter of application describing qualifications to **Dr. John Eppig, The Jackson Laboratory, Bar Harbor, ME 04609 USA.** Three confidential letters of reference should also be provided.

(NW4753)A

Chair, Department of Biochemistry

TEMPLE UNIVERSITY SCHOOL OF MEDICINE

The search for the Chair of the Department of Biochemistry has been extended. Candidates should have proven leadership ability and must be nationally recognized for their scientific contributions to biochemistry or related fields. The successful candidate must have demonstrated interest and ability in effective teaching and the capability of providing the administrative leadership to continue and extend the department's established strengths in research, teaching and service. An excellent opportunity exists to expand an already strong department and its collaborations with other Basic Science departments, as well as with the Fels Research Institute for Molecular Biology and Cancer Research, and the Thrombosis Center. Applications will be accepted until 15 February 1990 and should include curriculum vitae, a summary of current research, a description of teaching and administrative experience, and the names, addresses and telephone numbers of three or more references. Mail applications to: **Helen R. Buckley, Ph.D, Chair of the Search Committee, Department of Microbiology and Immunology, Temple University School of Medicine, 3400 North Broad Street, Philadelphia, PA 19140.**

Affirmative Action/Equal Opportunity Employer.

Choose (NW4756)A

TEMPLE UNIVERSITY

CT BIOMED BAD DEYNHAUSEN

KNOWLEDGE ENGINEER

The "Center of Technology Transfer Biomedicine" is a new Institute founded in collaboration with the University Bielefeld, located at a Medical Center in Ostwestphalia and a spa with campus like surrounding.

The "Division Expert Systems Biomedicine", invites a qualified person (Ph.D. or relevant experience in the field of biomedical sciences) to participate in the area of knowledge based systems. Salary depending on qualification: Pound 15.000-20.000. Native English speaker and knowledge in German required.

Further details: **Dr. B. Petkoff, Chief, Expert Systems Biomedicine, ZTB GmbH Bad Oeynhausen, W. Germany 4970,** Phone: 49-5731 9929. Equal Opportunities Employer.

(W6867)A

UNIVERSITY OF REGENSBURG, FRG

5 YEARS PROFESSORSHIP (C3) IN CELLULAR NEUROBIOLOGY

Applicants should be qualified in neurobiological research at the cellular level and adequately familiar with modern methods. Cooperation within resident Special Research Programme on Sensory Systems is requested.

Candidates must be qualified for temporary professorship by Habilitation or equivalent qualifications as senior scientist.

C.V., list of publications, and summary of research achievements should be sent to the **Dean, Faculty of Biology and Preclinical Medicine, University, Universitätsstr. 31, D-8400 Regensburg, FRG until January 30, 1990.** (W6848)A

THE UNIVERSITY OF MELBOURNE

RESEARCH FELLOW GRADE 1 (EXTENDIBLE TENURE)

CELL BIOLOGY FERTILIZATION IN PLANTS

School of Botany

A postdoctoral research position is available for studies of cell-cell interactions during fertilization in seed plants as part of a three year project funded by the Australian Research Council led by Dr. J. M. Pettitt, Department of Pathology and Immunology, Monash Medical School, and Professor Bruce Knox, School of Botany, University of Melbourne. Candidates should have a Ph.D. with experience in monoclonal antibody technology, protein chemistry or molecular biology.

The position is available for three years subject to the continued funding by A.R.C.

Further information concerning the position or the department may be obtained from Professor R. B. Knox, telephone (613) 344 5063 or FAX No. (613) 344 7049.

Salary is in the range: $A27,953-$A31,808 per annum (Research Fellow Grade 1).

Closing Date: 3 January 1990.

Position Number: 606A075

Written applications should reach The Director, Personnel Services as stated below.

Applications, in duplicate, including names, addresses and fax numbers of at least three referees and quoting the relevant position number should be addressed to The Director, Personnel Services, The University of Melbourne, Parkville, Victoria, 3052, Australia.

An equal opportunity employer. (W6849)A

HENDRIX COLLEGE
Biology Department
Two faculty positions:

(1) Developmental Biologist

to teach zoology, anatomy and embryological development;

(2) Microbiologist

to teach general microbiology and advanced course(s) in candidate's speciality. Both positions are tenure-track assistant professor, entry-level, and require participation in teaching introductory biology, direction of undergraduate research (start-up funding is available), student advising, and faculty governance.

Apply by February 1, 1990 to: **Dr. Bruce Haggard, Chairman, Biology Department, Hendrix College, Conway, AR 72032.**

An Equal Opportunity/Affirmative Action Employer (NW4736)A

SUNDERLAND HEALTH AUTHORITY

Leukaemia Lymphoma Resear
Laboratory

POST DOCTORAL RESEARCH ASSISTANT

Required from January/Februa 1990 to fill vacancy arising fro present incumbent gaining post U.S.A. Appointment initially f 1 year, renewable subject to pe formance. This is a spacious, we equipped laboratory, generous funded.

Experience in culture, stim lation and functional analys of human blood mononucle cells essential and familiarity wit ELISA techniques expecte Current research includes stud of immune consequences c blood transfusion, immuno pathology of Hodgkin's and othe lymphomas.

Salary on scale £10,458 £16,665, according to experienc and qualifications. Drive, initia tive and enthusiasm are essentia and will be rewarded. For furthe details/visit to department, tel 091 5656256 ext. 248. (Dr Kay).

We aim to be an Equal Oppor tunities Employer. (2298)A

RESEARCH ASSOCIATES IN REPRODUCTIVE BIOLOGY

UNIVERSITY OF MISSOURI

Two postdoctoral positions for recent Ph.D.s with experience in biochemistry, molecular endocrinology, or reproductive physiology to study either the biosynthesis, expression and function of alpha-interferons and 73 related lymphokines produced by mammalian embryos or the progestational control of protein synthesis and secretion in the mammalian uterus. Submit curriculum vitae and names of three references to **Dr. R.M. Roberts, 158 ASRC, University of Missouri, Columbia, MO 65211.**

(NW4752)A

MOLECULAR AGING

Two postdoctoral positions now available for persons trained in molecular techniques relevant to characterizing novel senescent-related genes and their cognate proteins. Support available for two or more years. Salary commensurate with experience. Send CV and names of four referees to **Dr. S. Goldstein, University of Arkansas Medical School and Veterans Hospital, 151 Research, 4300 West 7th Street, Little Rock, Arkansas, 72205,** tel. (501) 660-2069; Fax (501) 661-1202, ext. 3980.

(NW4747)A

BIOLOGICAL NMR SPECTROSCOPISTS

The Pittsburgh NMR Center for Biomedical Research is searching for one or possibly two Ph.D.-level scientists with experience in applications of NMR spectroscopy and imaging to metabolic studies in animals. These are full-time staff appointments and salary depends on experience and qualifications. Responsibilities include collaborative interaction with medical scientists, probe construction, and development and implementation of new NMR techniques. The Center is multidisciplinary involving scientists from both the University of Pittsburgh and Carnegie Mellon University. The Center houses a 4.7-Tesla, 40-cm Bruker BIOSPEC II and a 2.4-Tesla rapid imaging spectrometer is under construction. In addition, center personnel have access to a 7.0-Tesla, 15-cm bore Bruker BIOSPEC II and Bruker AM-300 and AM-500 spectrometers.

Applicants should send a Curriculum Vitae and have three letters of recommendation sent to: **Dr. Chien Ho. Pittsburgh NMR Center for Biomedical Research, Carnegie Mellon University, 4400 Fifth Avenue, Pittsburgh, PA 15213.** Review of applications will begin on February 15, 1990 and will continue until suitable candidates are recruited. Carnegie Mellon University is an Affirmative Action/Equal Opportunity Employer. (NW4745)A

Project Investigator

who will conduct independent research in the isolation of total RNA and poly(A+)–RNA. Construction, screening and expression of cDNA libraries. Molecular cloning. DNA sequencing. Northern and Southern blotting. Recombinant DNA methods. Purification and characterization of proteins. Tissue culture of mammalian cells. Ph.D. Degree in Biochemistry/Molecular Biology or Related Field. One year Research Training. One year Molecular Biology Research Experience. A minimum of 3 publications in the area of Molecular Biology and in particular in construction, screening, and expression of cDNA libraries and protein purification. 40 hours/week. $21,800 annual salary. Contact **Texas Employment Commission, Houston, Texas** or send resume to **Texas Employment Committion, TEC Building, Austin, Texas 78778.** Job Order #5757185. Ad Paid for by an Equal Opportunity Employer. (NW4763)A

UNIVERSITY OF TASMANIA

Applications are invited for suitably qualified men and women for the following position.

RESEARCH FELLOW IN PHYSICS

(Ref. 165/89)

The Department of Physics requires a theoretical physicist/mathematical physicist to work on a joint University of Tasmania/University of Queensland Australian Research Council funded research project on infinite dimensional algebras and field theory applications. Candidates should have a Ph.D. and relevant research experience.

The position is available for up to three years, and the appointment will be in the Junior Research Fellow scale (A$27,953 to A$31,808 per annum), with the possibility of assistance with removal expenses.

For further information contact Dr P.D. Jarvis, tel. (61.02) 202401, or Dr M.D. Gould, tel. (61.07) 3772673.

Applications quoting the reference number and including curriculum vitae and the names and addresses of three referees should be addressed to reach the Staff Officer, University of Tasmania, G.P.O. Box 252C, Hobart, Tasmania 7001 by 9 January 1990.

The University is an Equal Opportunity Employer. (W6842)A

Molecular biologist

Position open for a molecular biologist. Research project includes demonstration of the gene, and receptor expression and the role of cytokines in human endometrium. Send CV to **Dr. Tabibzadeh, Dept of Pathology, Elmhurst Hospital, 79-01 Broadway, Elmhurst, NY 11373 or call: 718-830-1685.** (NW4755)A

POSTDOCTORAL POSITION

Available for investigating regulation of cell-to-cell communication via gap junctions. Experience with protein chemistry and molecular biology or patch clamp methods preferred. The position is available for up to 3 years with possible extension.

Send CV and 3 letters of reference to **Dr. Camillo Peracchia, Department of Physiology, Box 642, University of Rochester, Medical Center, Rochester, New York 14642, USA.** (NW4728)A

BIOSCIENCE WRITER

with business reporting experience, some computer (Macintosh) friendliness, for position as Associate Editor on periodical of major N.Y. publisher. Salary, plus perks, commensurate with qualifications. [An equal op. employer]. Resume to **Box No. NW4760, c/o Nature, 65 Bleeker St., New York, NY 10012.** (NW4760)A

STANFORD UNIVERSITY PSYCHOLOGY DEPARTMENT

is seeking applicants for a professorship at any level in Physiological Psychology starting Autumn 1990. Scholars with a strong research record in sensory processes or the biological basis of cognition or behavior are encouraged to apply. Send curriculum vitae, copies of scholarly papers, and three letters of recommendation to: **Physiological Search Committee, c/o Mrs. Frances Martin, Dept. of Psychology, Stanford Univ., Stanford, CA 94305-2130** by February 15, 1990. *Stanford University is an Equal Opportunity Employer.* (NW4764)A

STUDENTSHIPS

UNIVERSITY OF CAMBRIDGE

Research Opportunities in Physical Chemistry and Chemical Physics

Applications are invited from students expecting good Honours degrees in Chemistry, Physics or Materials Science for studentships which will become available from October 1990 in the following areas:

☆ ATMOSPHERIC CHEMISTRY
☆ GAS PHASE KINETICS AND PHOTOCHEMISTRY
☆ HETEROGENEOUS CATALYSIS
☆ SOLID STATE CHEMISTRY
☆ SPECTROSCOPY
☆SURFACE SCIENCE
☆ X-RAY DIFFRACTION AND ELECTRON MICROSCOPY

CASE and Quota SERC Studentships as well as fully funded industrially supported Studentships are available. Further details and information concerning visits to the Department may be obtained (quoting reference RS/NT) from **Professor D. A. King, Department of Chemistry, Lensfield Road, Cambridge CB2 1EW.** (2352)F

UNIVERSITY OF OXFORD

RESEARCH STUDENTSHIPS

The Department of Human Anatomy invites applications for several research studentships in 1990, including the possibility of a Wellcome Trust Prize studentship. The department is well endowed with modern research facilities and at present research interests cover the following topics: neuroscience, endocrinology, membrane physiology, developmental and cell biology. Detailed information on these research programmes can be obtained from the Department.

Applicants, who should have or expect to be awarded a first class or upper second honours degree in one of the biological sciences, should send a copy of their curriculum vitae, a statement of their research interests, and the names of two referees to: **Professor R W Guillery, FRS, Department of Human Anatomy, South Parks Road, Oxford, OX1 3QX, by 15 January 1990.** (2341)F

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AWARDS

NASA PLANETARY BIOLOGY INTERNSHIPS

The Marine Biological Laboratory, Woods Hole, MA, invites applications from graduate students and seniors accepted to graduate programs for awards of $2000 plus travel to participate in research at NASA centers and collaborating institutions for appoximately 8 weeks. Typical intern programs include: global ecology, remote sensing, microbial ecology, biomineralization, origin and early evolution of life. Application deadline: 1 March 1990.

For information/applications, contact: Greg Hinkle, Planetary Biology Intern Program, Department of Botany, University of Massachusetts, Amherst, MA 01003.

An Equal Opportunity/Affirmative Action Institution. (NW4737)N

THE HEART RESEARCH INSTITUTE, SYDNEY, AUSTRALIA

RESEARCH FELLOWSHIPS AND ASSISTANTSHIPS AVAILABLE IMMEDIATELY

The Heart Research Institute is a new dynamic National Institute, comprising five major groups with interests in cell biology, immunology, molecular biolcgy, biochemistry and coagulation with particular emphasis on mechanisms of atherogenesis. Several research fellowships and assistantships are available at the Heart Research Institute as a result of recent successes in funding applications to the Australian Research Council, the National Heart Foundation, the National Health and Medical Research Council, the New South Wales Cancer Council and other bodies. Applications are invited in the following areas:

1. Effects of thermal stress on the release of coagulation-associated molecules by human endothelial cells: Dr. Joan Dawes. Relevant Reference: Human Pathology, Vol. 18, 234 (1987).
2. Free radical damage to proteins and the roles of protein hydroperoxides as reactive intermediates (3 positions: Prof. Roger Dean). Relevant References: Biochem J. 250, 87 (1988); 256, 205 (1988); Analytical Biochemistry 176, 353 (1989).
3. Glycosaminoglycan degradation by macrophages and the possible role of glycosaminoglycan fragments in atherogenesis: Prof. Roger Dean. Relevant Reference: Bioscience reports, 4, 1017 (1984).
4. One Molecular Biologist and one Cell Biologist to study a newly described chemotactic factor in inflammation: Dr. Carolyn Geczy. Relevant Reference: J. Immunol. 141, 2110 (1988).
5. Oxidation of low density lipoprotein and its influence on atherogenesis. Dr. Wendy Jessup. Relevant Reference: Biochemical Journal 262, 707, (1989).
6. Roles of metallothionein in protection against free radical-mediated cardiotoxicity by adriamycin: Dr. Jeremy Simpson. Relevant References: Toxicology 53, 231, (1988); Biochemical J. 250, 87 (1988).
7. Molecular mechanisms of endocytosis with special reference to the LDL receptor related protein: Dr. Keith Stanley. Relevant Reference: Nature 341, 106, (1989).
8. Mode of action and interaction of antioxidants: Dr. Roland Stocker. Relevant reference: Science 235, 1043 (1987).

Most positions are available for two or three years with the possibility of continuation. The position associated with Project 6 is a part-time position for one year in the first instance. Salaries are in accordance with NH&MRC scales. Further information is available on request to the named members of the staff of the Heart Research Institute. Written applications should be made to:

Professor Roger Dean, Director, Heart Research Institute,
145 Missenden Road, Camperdown, Syndey, NSW 2050
AUSTRALIA
including c.v., publications list and the names of two referees.
Phone: **+61 (2) 550 3560, Fax: +61 (2) 550 3302.**

(W6844)E

FELLOWSHIP IN NEUROIMMUNOLOGY

Position is available for post-doctoral Fellowship in Neuroimmunology. Candidate should have Ph.D., or M.D./Ph.D. degree. Research experience in Immunology for a minimum of 2 years is essential. Candidates with experience in molecular biology will be preferred.

Please send curriculum vitae and three letters of recommendation to: **Sudhir Gupta, M.D., University of California, Irvine, C264A Med Sci I, Irvine, CA 92717.** Telephone: (714) 856–5818.

UCT is an Equal Opportunity and Affirmative Action Employer.

(NW4741)E

UNIVERSITY OF GLASGOW
POSTDOCTORAL FELLOWSHIP
in Molecular Neurobiology

A 3-year appointment supported by the Medical Research Council is available for work on cloning and functional analysis of genes characteristic of a particular class of mouse CNS neurone, in the laboratory of Professor R. Wayne Davies. Candidates should have good practical experience in recombinant DNA technology, and will have the opportunity to learn transgenic and chimaeric mouse technology. The laboratory is in the new Robertson Institute for Biotechnology within the Department of Genetics which is active in many fields ofmolecular biology research.

Salary will be within Research Assistant 1A scale £10,458-£12,879 per annum.

Further particulars may be obtained from **Professor R. Wayne Davies, Robertson Institute for Biotechnology, University of Glasgow, Glasgow. G12 8QQ** (041 339 8855 ext. 5102), to whom a letter of application with full c.v. together with the names and addresses of three referees should be sent on or before 9th February, 1990. (2339)E

COURSES & CONFERENCES

ÉCOLE EUROPÉENE DE BIOLOGIE THÉORIQUE: EBT90

Abbaye de Solignac, France. 2–22 Septembre 1990

Title: Structures and functions in biological organizations (theoretical basis for modeling and simulation).

Aims: Complexity in biological organizations necessitates the development of particular methods (modeling and simulation); experimental biology suffers from a lack of communication between life sciences and physics and mathematics. So, although experimental methods within these sciences may be different (there is no homeostasis in physics systems but homeostasis is the basis of the definition of experimental procedure validity in life sciences), the structure of the processes (unstable state typology, spatial heterogeneity distribution, dynamics structures — oscillations, chaos, limit cycle . . .) are likely to be described by the same types of formalisms. Theoretical biology utilizes this property to build new methods to answer the question of complexity in biological organizations.

Maximum number of participants: 50.

Languages: Courses and lectures in French or English depending on speaker; supporting documents (courses and lectures texts) will be available.

Programme: First week: optional courses (33 hours); second and third weeks: lectures (63 hours)

— *optional course "biology":* General biology, Symetry and polarity, Main functions and controls (animal physiology), Morphogenesis and full plant physiology, Genetics and evolution, Evolution and diversity, From ecosystem to biosphere.

— *optional course "Mathematics":* Basic linear algebra, Differential equations, Stochastic modelling and statistical approach, Fundamental physics: the main formalisms, Introduction to PDE-modeling, Recurrent equations, Qualitative dynamics.

— *Lectures:* Epistemology, Singularities and catastrophs, Dynamical systems, System sciences and cybernetics, Physics, Form analysis, Automata, Theoretical computering, Neurology, Electrophysiology, Genetics, Metabolic networks, Morphogenesis, Ecological dynamics.

Cost: 1180 EEC ecus (*including accommodation and documents*).

EBT90 is organized by the Société Française de Biologie Théorique *with the support of the INRA Formation Permanente office.*

EBT90 is granted by the Commission des Communautés Européennes and by the Conseil Régional du Limousin.

Informations and detailed programme:

Dr. A. Kretzschmar, INRA, B.P. 91, 84140 MONTFAVET - France.

Registration form (to be requested before 28 February 1990):

Formation Permanente INRA (Att. Pascaline Garnot), 145 rue de l'Université, 75341 PARIS Cédex 07 - France.

(W6859)C

CELL DIFFERENTIATION/ONCOGENESIS

INTERNATIONAL CONFERENCE SPONSORED BY THE EUROPEAN TISSUE CULTURE SOCIETY AND THE INTERNATIONAL CENTER FOR CANCER RESEARCH

MAY 4-7, 1990, ATHENS, GREECE

TOPICS:

1. Cell interaction
2. Matrix interaction
3. Growth and differentiation factors
4. Differentiation of cancer cells
5. Teratocarcinomas
6. Hemopoietic differentiation
7. Neuronal differentiation
8. Oncogenes, onco-suppressor genes and differentiation

Enquiries and Registration
Professor Demetrios A. Spandidos
National Hellenic Research Foundation
48 Vas. Constantinou Ave.
ATHENS 116 35, GREECE
(Tel. 7241505 and 7210274) (Fax 7212729)

(W6864)C

INDUSTRY, UNIVERSITIES AND WEALTH CREATION

Worldwide Trends and Opportunities for Europe

Queens' College, Cambridge
4-6 July 1990

This international conference will bring together industrialists and academics from Europe, USA and Japan, to explore collaboration between companies and universities, especially in research and new business generation.

Authoritative perspectives will be presented by speakers prominent in their fields, for example, Sir Geoffrey Allen (Unilever), Professor P Fasella (EC, DG XII), Professor H Fusfeld (USA).

The intention is to highlight opportunities for Europe and to stimulate specific action to be taken up by European companies and universities.

Designed to interest senior industrialists, academics and government officials who are decision-makers and leading players in the European scene, the main headings for the 4 sessions of this 2½-days conference programme will be:–

1. International Perspectives: strategic approaches in Europe, the USA and Japan
2. Case material illustrating worldwide experiments in industry/university partnerships in research
3. Industry/Higher Education interaction in the development of new business.
4. Opportunities and the way ahead for Europe.

Those who have had experience of practices in this field in America and Japan will also be welcome.

For further details, please contact:–

University of Cambridge
Wolfson Cambridge Industrial Unit
20 Trumpington Street
Cambridge CB2 1QA, UK
Telephone: (0223) 334755
Fax: (0223) 332662

(2357)C

SEMINARS & SYMPOSIA

The Third International Karolinska Institute Center for Biotechnology Symposium

Intracellular Routing of Protein Molecules

Huddinge, Stockholm, May 18-19 1990

Topics

Mechanisms of Membrane Transiocation – Protein Maturation and Sorting in the Secretory Pathway – Mechanisms of Vesicle Traffic

Speakers

Robert Freedman (GB), Ari Helenius (USA), Richard Klausner (USA), Ben de Kruijff (NL), David Meyer (USA), Walter Neupert (FRG), Peter Novick (USA), Sjur Olsnes (Norway), Barbara Pearse (GB), Linda Randall (USA), Tom Rapoport (GDR), James Rothman (USA), Kai Simons (FRG), David Vaux (FRG), Bill Wickner (USA).

Organizing committee

H. Garoff, G von Heijne, P. Liljeström, M. D. Baron

For information and application forms, please contact:

The Third International CBT Symposium,
Dept. of Molecular Biology
NOVUM, S-141 52 Huddinge, Sweden.
Phone: Int+46-8-779 52 35. Fax: Int+46-8-774 55 38

DEADLINE FOR APPLICATION IS FEBRUARY 15th, 1990

(W6854)M

continued on page 16

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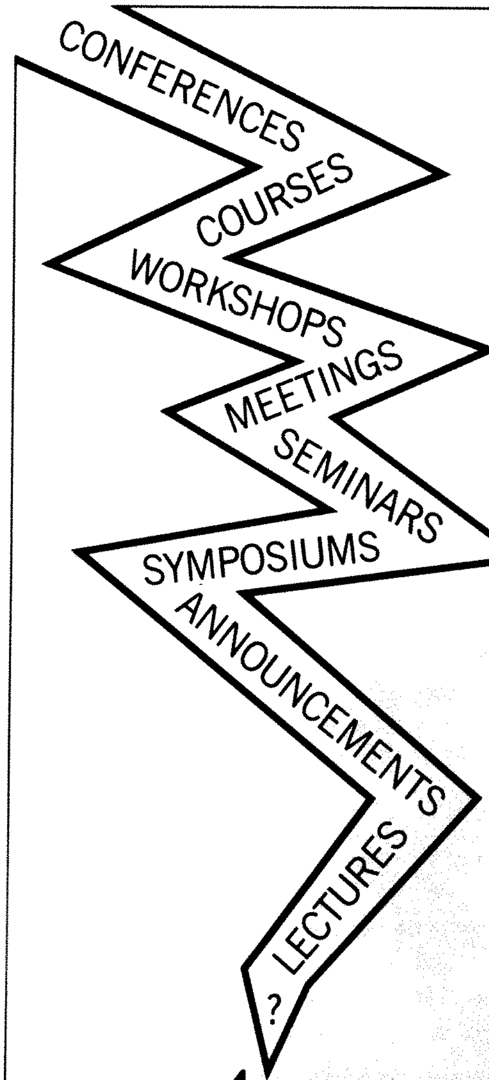
(W6861)B

MISCELLANEOUS

KNOWLEDGE is one bit (of variable strength) per synapse. Fo reinforcement to work it must be delivered to the correct synapse (or failing that the correc neurone). This can be bes achieved if there is a binary enumeration of synapses (or failing that of neurones). The homeobox deletions, the cell lineages of caenorhabditis and the symmetry of vitiligo can all be interpreted as evidence of the enumeration of biological structures. — Box No. 2338, c/o Nature, 4 Little Essex St., London WC2R 3LF.

(2338)J



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Call for Papers

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Editor-in-Chief: W. French Anderson, M.D.

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As a major new frontier, gene therapy deserves the focused attention of its own publication. Since human gene transfer has been approved for clinical trials, we are at the threshold of an era where this new therapeutic approach will probably be used in the treatment of presently incurable diseases. Several therapy protocols are being tested in preclinical animal trials. Cancer, AIDS, and genetic diseases are the initial targets.

Original scientific contributions will include:

- ***Techniques for gene transfer into mammalian cells***
- ***Construction and testing of retroviral vectors***
- ***Examination of the life cycle of retroviruses where it is applicable to the use of retroviral vectors***
- ***Regulatory mechanisms for controlling gene expression***
- ***Animal models for human diseases***
- ***Studies evaluating gene transfer and expression in animals***
- ***Results of preclinical safety and efficacy studies***
- ***Results of clinical studies***

Other topics in *Human Gene Therapy* will include ethical, legal, regulatory, social, and commercial considerations.

Human Gene Therapy will address itself to all aspects of this specific field including broader social issues; additionally, this important new publication will serve as a resource for current information as well as a repository for future reference.

Submit manuscripts to the Editor-in-Chief:
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Bldg. 10, 7D-18
National Heart, Lung and Blood Institute
National Institutes of Health
Bethesda, Maryland 20892
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(NW4735)G

Biochemical Endocrinology Symposium

The Twelfth Annual University of California, Riverside-Nichols Institute Symposium on Cellular and Molecular Endocrinology will be held Thursday and Friday, March 29 and March 30, 1990 on the University of California, Riverside campus at the foot of the Box Springs Mountains.

The purpose of the meeting is to foster communication among scientists who are interested in the cellular, biochemical and structural aspects of endocrinology. The meeting will have four sessions devoted to the topics of (a) Control of Cell Growth, (b) G-Protein and Regulation of Ion Channels, (c) Regulation of Gene Expression and (d) Animal Disease Models.

Thirteen internationally recognized scientists will present lectures on their work in these areas. They include the following: Donald M. Bers (Riverside), Ralph A. Bradshaw (Irvine), Arthur M. Brown (Houston), Richard Cate (Cambridge), Susanna Cotecchia (Durham), Sidney Fleischer (Nashville), Richard D. Klausner (Bethesda), Jeffrey A. Ledbetter (Seattle), Donald P. McDonnell (Houston), John W. Newport (San Diego), Paul M. Quinton (Riverside), Timothy A. Stewart (South San Francisco), and David O. Toft (Rochester).

Registration fees for the meeting are $25.00 per participant ($15.00 for postdoctoral students/fellows; $10.00 for students and $125.00 for physicians wishing to enroll in the Continuing Medical Education [CME] Program [Category 1] through the University of California, Los Angeles School of Medicine).

For registration and further information please write: **Dr. Anthony W. Norman, Endocrinology Symposium, Division of Biomedical Sciences, University of California, Riverside, CA 92521; telephone: (714) 787-5705; FAX: (714) 787-5504.**

(NW4734)M

Cancer Research Campaign

THE ROLE OF CYTOKINES IN CANCER

A National Symposium in Oxford

29th March, 1990

Organized by C.E. Lewis & J. O'D McGee

Speakers include:

F. Balkwill (London)	**D. Cantrell** (London)
G. Gallagher (Strathclyde)	**P. J. Guillou** (London)
A. Harris (Oxford)	**C. E. Lewis** (Oxford)
R. Rees (Sheffield)	**M. G. Wing** (Cambridge)

– Admission Free –

For further details and booking forms etc. contact **Miss Lesley Watts, Nuffield Dept. of Pathology & Bacteriology, John Radcliffe Hospital, Oxford 817834.**

Numbers limited – preference given to applications from UK Labs. Final deadline for applications: Jan. 22nd, 1990.

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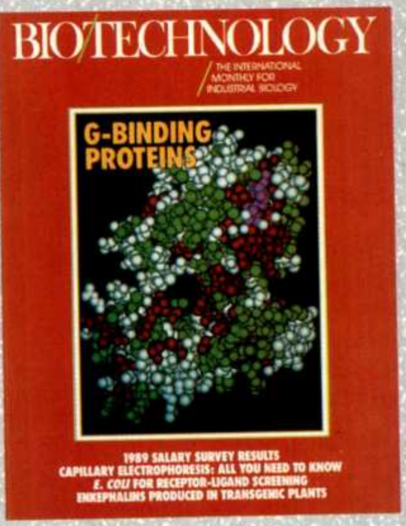
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